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PRACTICAL EXERCISES

IN

PHYSIOLOGY

BY

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WITH THE CO-OPERATION OF

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PREFACE.

THE following exercises are intended to serve as a guide to the Practical Courses which are given in the Physiological Department of this College. Part III. comprises the Chemical Exercises relating to Food Stuffs and Animal Liquids which are performed by every Student in the Class Room, in the ordinary course of Practical Physiology. These Exercises were, for the most part, originally arranged by Mr. Page. They have been used by many hundreds of Students and have been found to work well. Part IV. contains directions for the more detailed practical study of the same subjects. In the preparation of these, I have had the assistance of Mr. North who has used them in the Practical Instructions which he has given here. The exercises in Part I. relate to the Physiology of Muscle and Nerve. In selecting them great care has been taken to include nothing which cannot be successfully carried out by the Student. Many of the exercises have been contrived by Dr. Augustus Waller, who has de-

voted much time and thought to the simplification of methods. The Demonstrations in Part IV. relate to various subjects. These are separated from the rest of the course, on the ground that they do not admit of being performed by each student for himself.

UNIVERSITY COLLEGE COURSE

OF

PRACTICAL EXERCISES IN PHYSIOLOGY.

PART I.

PRACTICAL EXERCISES RELATING TO THE PHYSIOLOGY OF MUSCLE AND NERVE.

1. *Make electrodes as follows:*—Prepare two straight, moderately thick wires about four inches long. Taper each to a blunt point at one end. Solder to the opposite end of each a length of thin wire. Cover each with a thin layer of packing wax. Prepare two three-inch lengths of glass tubing (which should be thick walled and of narrow bore). Warm them, and introduce the wires so that their points project half an inch. Bind the tubes together for convenience of handling and bare the wires by scraping the wax off near the point on one side.

2. *Put up a Daniell cell.* The positive element is a well amalgamated zinc rod immersed in ten per cent. sulphuric acid contained in a porous cell; the negative element is a copper cylinder containing a solution of sulphate of copper. Put a wire in each binding screw. The end of the wire attached to the zinc (negative wire), is called the cathode; that attached to the copper (positive wire), the anode.

3. *Pith* a frog and prepare a sciatic nerve without dividing it,* (See Hdb. p. 343). In the process the gastrocnemius should not have twitched.

4. *Connect the electrodes with a Daniell cell, interposing a key in the circuit.* Contraction follows make, or make and break. It does not continue during passage of current. The excitability of the nerve is increased by division or injury.

5. Arrange cell and coil for single shocks, *i.e.*, join the ends of the battery wires to the two top screws of the du Bois' induction apparatus, in which the primary wire ends, interposing a key by which the current is made and broken at will. Gradually sliding the secondary towards the primary coil, observe that the break shock is first responded to, then the make. Note the distance of secondary from primary coil at which you first get contraction in each case.

6. Arrange cell and coil for repeated shocks (faradisation), by bringing the battery wires to the two screws *A* and *E*, Fig. 1. The circuit now includes the vibrating hammer or automatic interrupter. On closing the current, the hammer is drawn down and causes a break; the current ceasing, the hammer is released, and contact is restored by the spring. You thus obtain a succession of make and break induction currents in alternately opposite directions. Prepare the second sciatic nerve, and observe that faradisation produces continuous muscular contraction or tetanus, which may be due either to the series of break excitations, or to the double series of strong break excitations and weaker ones at make. Note the distance at which you first get contraction.

7. To obtain successive make and break excitations of nearly equal intensity, arrange for single shocks as in 5.

* In future experiments it is assumed that pithing is performed as a matter of course.

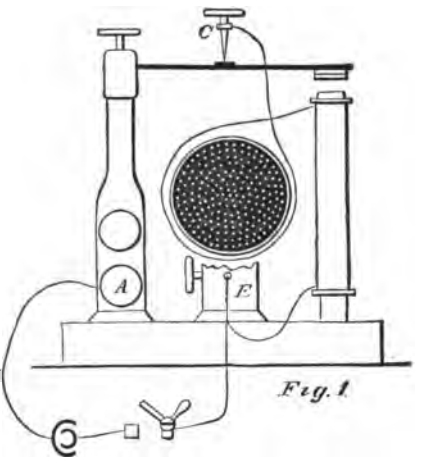


Fig. 1

But in addition, connect the two ends of the primary coil (the top binding screws), by an extra derivation or "short circuiting" wire broken by a key. By closing this key, the current in the primary coil is diminished by derivation, and increased to the same amount when it is opened. The diminution and increase give rise to induction currents, of which, the directions are opposed like those produced by make and break. Both of them are *cæt. par.* weaker than the make induction current, and they are sensibly equal to each other in their excitatory effects.

8. **Helmholtz' Modification of the Induction Apparatus.**—When it is necessary in faradising that the excitatory effects of the make and break shocks should be equal, the apparatus is arranged as in fig. 2. Connect the battery wires as in 6. Bridge the interrupter by a wire extending from B to C. Raise the upper contact screw C out of reach, and bring the

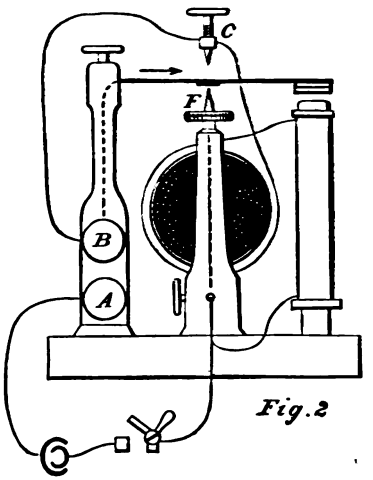


Fig. 2

lower (F) within reach of the spring. Here, as in the other case, the current in the primary coil diminishes by derivation, when the descending hammer touches F; increases to the same amount when it rises, but is never broken.

9. *Use of the du Bois key.* Before proceeding further, note that a key may be used for throwing a current into or cutting it off from a nerve or other excitable structure in two ways, viz., (1) in such a way that when it is closed the current is made, when it is opened the current is broken; or (2) so that when the key is closed it acts as a bridge, by which so large a proportion of the current is derived, that it in effect vanishes in the part of the circuit beyond the bridge.

In using induction currents for excitation, always employ the second method.

10. **To Cut off the Make or Break Shock.**—For this purpose a key may be introduced into the secondary circuit, by

which it can be closed or opened at will during make or break of the primary current; or this may be effected automatically, by fixing an ebonite rod made for the purpose to the hammer, so as to prolong it for about an inch. The rod carries at its end a platinum wire in the shape of an inverted U. The two limbs of the ∩ are of such length that they dip into two pools of mercury, which are severally connected with the ends of the secondary coil. Consequently, when the hammer descends, the ∩ bridges the two pools, so that the secondary coil is short circuited. As at the moment of break of primary circuit the hammer is down, the break shock is thus cut off. If the pools, instead of being connected as above, are interpolated in secondary circuit, the make shock is cut off.

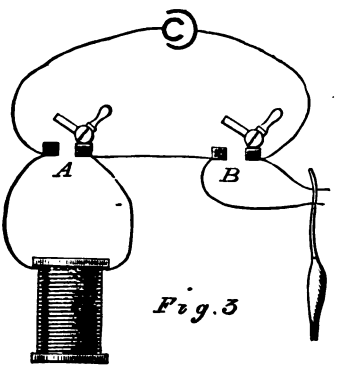


Fig. 3

11. **Physiological Proof of the Break Extra Current.**—The extra current is the current produced in a coil by the inductive influence of contiguous turns on each other, when a voltaic current commences or ceases through the coil; its direction is *against* that of the battery current at *make*, *with* it at *break*. Establish connections, as in Fig. 3, placing the

electrodes on the tongue. Close the key, *A*, so that the current is cut off from the coil. Observe that opening and closing the current by the key, *B*, produces little or no appreciable effect. Now open the key, *A*, so that the current passes through the coil. Opening the key, *B*, gives rise to a strong effect which is due to the break extra current.

12. Introduce a second pair of electrodes into the current of the battery and primary coil, arranged as in 5, and apply them to the tip of the tongue. Observe the effect of making and breaking the current, first with, and then without the core. Similarly compare these effects with those of the induced make and break currents in the secondary wire.

13. **Unipolar Excitation.**—Connect one electrode with the secondary coil, and apply it to the nerve. If the preparation is completely insulated, there should be no response to make or break. If the insulation is destroyed by touching the preparation, or otherwise, contraction occurs.

It is to avoid unipolar excitation that, as a rule, the induction circuit is directed to be thrown in and out by using the key as a bridge (See 9). Unipolar excitation is more apt to occur with the break shock than with the make, in consequence of the greater intensity of the former. Consequently, it is avoided by using Helmholtz' modification. Prove this by experiment.

14. Make and record observations on the comparative excitability of nerve and muscle as follows:—Note the approximation (in centimeters) of the secondary to the primary coil which is required to obtain a response (1) to the make induction shock; (2) to the break; (3) to faradisation, with the nerve undivided. Then repeat the observation after dividing the nerve. Compare these effects with those observed when the electrodes are applied directly to the muscle.

15. **The Ritter-Valli Law** (H.* p. 334). Prepare a sciatic nerve without severing it. Compare the excitability to faradisation of the different parts of the nerve, placing the electrodes (1) at the ischium; (2) close above the knee: In about an hour repeat the experiment, using the same nerve. Then sever the nerve near its origin, and so repeat the experiments. Note the results in each case. Finally, repeat the observations, using the nerve of the opposite limb.

16. **The Rheochord.**—Whenever very weak voltaic currents are required, we use the Rheochord. The simplest, and most convenient form is a long wire of German silver, of about 20 Ohms resistance, which, for convenience of space, is wound on glass pegs which are fixed at equal distances in two rows at opposite ends of a well-varnished mahogany board. The wire is then divided into as many equal lengths as there are pegs. On the board, underneath the first length is a scale, each division of which is $\frac{1}{100}$ of the whole length of the wire. The wire ends in two blocks, A and B, each of which has two binding screws. In use, the battery wires are connected with these two blocks. One of them (the one from the graduated end of the rheochord wire) also receives the wire of the second electrode. The other electrode wire is brought to a sliding block, by which contact can be established with the rheochord wire at any distance from A. In this arrangement, the current through the nerve, or other structure to which the electrodes are applied, is *proportionate to the length of wire between the slider and the block.* This relation would not hold good were it not that the resistance of the nerve is always very great as compared with that of the wire.

17. **Polarisation of Electrodes.**—Place a pair of electrodes under the sciatic nerve, and join them by a key, and satisfy

* In this and other similar references, H. stands for Hermann, and F. for Foster.

yourself that opening and shutting the key gives no contraction. Connect the two wires of a Daniell with each side of the same key, which, therefore, bridges the current. Allow the current to pass through the nerve for a few instants by opening the key, remove the battery wires, leaving the electrode wires attached to the open key. Close the key, the muscle will contract, the electrodes having been polarised by the previous current. Leave the key closed for a few instants; open and again close, the muscle will not contract, polarisation of the electrodes having subsided during closure of circuit. This gives a reason for using the key as a bridge to cut off the constant current. If, in experiments on the law of contraction (See § 32) it is used to make and break the current, it will be noticed that at each successive closure the contractions diminish as polarisation augments.

18. **Unpolarisable Electrodes.** (H, 287, F. 55). The form in common use consists of (1) a smooth, amalgamated* zinc rod dipping into (2) a saturated solution of zinc sulphate, with which the tissue is electrically continuous by (3) a plug of china clay made into a paste with saline solution ·75 per cent. Threads may be used to connect the plug with the tissue. Such electrodes are of high resistance. (For the method of testing them with reference to their freedom from polarity, See I., 17).

19. **Galvani's Experiment.**—Connect two dissimilar metals, *e.g.*, zinc and copper wire; and apply their points to a nerve, or one point to a nerve, the other to any part of the frog; contraction occurs at make, or if the preparation is very excitable, at make and break.

* To make amalgamating liquid for electrodes, dissolve with gentle heat 3 c.c. of mercury in a mixture of 50 c.c. nitric acid, and 150 c.c. of hydrochloric acid. Dilute this liquid for use with its own volume of hydrochloric acid, and eight times as much water.

20. **The Contraction without Metals.**—Prepare a nerve-muscle preparation, choosing a vigorous and lively frog. Lay the nerve on the muscles of the other limb stripped of its skin. The muscle of the preparation contracts because different parts of the surface with which the nerve is suddenly brought into contact are at different potentials.

21. **The Secondary Twitch.** (F. p. 59, H. 290). Prepare the sciatic nerve of one limb of a vigorous frog, and prepare a nerve-muscle preparation from the other limb. Strip the skin off the first limb, and lay the nerve of the preparation on the gastrocnemius. Apply various stimuli to the first nerve; both muscles contract; the secondary muscle contracts because its nerve is stimulated by the sudden electrical changes which accompany the contraction of the primary muscle.

22. **The Paradoxical Contraction.** (H. 343, 346). Dissect out one of the two main divisions of the sciatic, and divide it at its periphery. Galvanic excitation of the peripheral part of the divided branch, gives rise to contraction of the muscles supplied by the other branch. The second nerve is stimulated by the electrotonic alteration of the first nerve.

23. **Action of Curare.** (F. p. 38). *(a)* Inject one drop of curare solution (1 per cent.), having stopped the circulation of one limb by a ligature from which the nerve is excluded. In a few minutes, test muscle and nerve of both limbs. Both react on the ligatured side; on the other side, muscle reacts, nerve does not.

(b.) Proceed as before, but use the ordinary dose of curare, 1 to 2 drops of 0·1 per cent. solution, and wait longer. Inject 1 drop of strychnia solution, (·05 per cent.) Pinching or touching curarised limbs, which have lost motility, will excite reflex motion in the protected limb. Curare does not paralyse afferent nerves or spinal centres.

For these two experiments the frog is killed by destroying the hemispheres: the spinal cord must be left intact.

(c.) Make two preparations of muscles with nerves A and B. Of A allow the muscle to soak in curare dissolved in $\frac{1}{2}$ per cent. saline solution, keeping the nerve moistened with saline. Of B allow the nerve to soak in curare. In about 15 minutes, test muscle and nerve of both preparations; both muscles are excitable; the nerve of A is inexcitable, that of B is excitable. Curare does not paralyse motor nerve or muscle, but makes a block between nerve and muscle.

24. **Mechanical Excitation; Mechanical Tetanus.**—It has already been seen that section or other mechanical injury is an excitant of nerve, evidenced by one or more muscular twitches.* Connect a Grove cell with the "Tetanomotor," introducing a key into the circuit. The wire from the zinc terminal of the battery must be inserted in the binding screw marked *E*, that from the platinum in *Z*, in Fig. 1. Adjust the apparatus so that on closing the key the ivory hammer vibrates so as to excite, without destroying, a nerve placed on the ivory groove. The effect produced is identical with tetanus by faradisation.

25. **Experiments in which the Graphic Method is used.**—For these experiments are required, (1) a revolving cylinder tightly covered with paper, and then smoked, of which the rate of rotation is known; (2) suitable means for supporting a muscle, so that it may act upon a lever which presses lightly against the moving surface of the paper.

* The positive element is a well amalgamated zinc, immersed in ten per cent. sulphuric acid contained in a porous cell; the negative element is a sheet of platinum immersed in strong nitric acid.

By noting the time required for a given number of revolutions of the cylinder, and accurately measuring its circumference, the rate of motion of the surface which is to receive the record, may be fairly determined. But for more accurate purposes, we must make a simultaneous record of the oscillations of a tuning fork, or of an electro-magnetic time-marker, introduced into a circuit in which a vibrating reed acts as an electro magnetic interrupter.

When for the purposes of the experiment, it is desirable that the horizontal motion of the recording surfaces should be slow, *i.e.*, less then two inches per second, an interrupting clock regulated by a pendulum or a metronome, is substituted for the vibrating reed in the circuit of the time-marker. This is so constructed that it makes and breaks the circuit at intervals of one or more seconds.

26. **The Myograph.**—A simple and useful myograph is constructed as follows:—An oblong block of wood, on which a cork plate is glued, supports the preparation. At one end of the block is a vertical stem or pillar, of which the height and distance from the preparation can be varied. This pillar is surmounted by two steel points, which face each other at a distance of about three quarters of an inch. On these the axis of a bell-crank lever rotates, of which the horizontal arm is prolonged by a rod of wood or thin vulcanite, and ends in a writing point. To the vertical arm, the tendon of the muscle of which it is desired to record the contractions, is attached.

27. **The Graphic Record** or curve of a **Single Contraction, (twitch).** (F. p. 42, H. p. 270). *(a.)* First arrange the recording apparatus. For the present purpose the cylinder must revolve about once in two seconds. With the apparatus commonly used, a trigger key is provided, which can be so

adjusted that the cylinder opens it on arriving at a certain point in its revolution. See that this key is in order, particularly that when it is closed the contact is perfect. Cover the cylinder with glazed paper, and smoke it over a petroleum lamp. Cut off the hind limbs of a frog just killed by pithing, and sever them from each other. Place one of them on the cork plate, in such a position that the tibia is in a line with the long arm of the lever. Expose the tendon of the gastrocnemius and after severing and freeing it from surrounding parts, tie to it a bit of strong ligature thread immediately in front of the sesamoid cartilage. Then expose the lower end of the femur, and thrust through it a strong needle-point, to which a thin wire leading from one of the binding screws at the side of the block has been soldered. This serves to fix the femoral attachment of the gastrocnemius. The needle in which the wire from the other binding screw ends, is to be thrust through the tendon close to the ligature. Lastly, attach the thread to the short vertical arm of the lever, bring the myograph (which has up to this time been on the table), into position, connect its binding screws with the wires of the secondary coil of the induction apparatus, which should be at a considerable distance from the primary. The next step is to ascertain to what distance it is necessary to approximate the secondary coil, in order to obtain a full response to break induction shocks. This having been determined, open the key in the primary circuit, bring the end of the writing lever into contact with the smoked surface, ascertain that the trigger key is in position, close it and allow the cylinder to revolve slowly until the pin presses against the trigger. Tap the tendon lightly, and set the clockwork in unrestrained motion. As soon as the fly has attained its full expansion, momentarily close the key in the primary circuit.

(b.) **Influence of Temperature on the Form of the Curve.** The prolongation of the single contraction produced by cold, may be observed by placing a few bits of ice in contact with the skin by which the muscle is covered. To study the influence of heat, remove the ice, and place on the limb an India-rubber bag filled with warm water; you find that the contraction is shortened. If the water is above 40° C., the muscle may become rigid.

(c.) **Influence of Veratrin.**—Inject a drop of 0·1 per cent. solution of veratrin into the lymph sac of a brainless frog. After twenty minutes, destroy the spinal cord and inscribe one or more muscle curves, and compare them with those previously obtained.

28. **Superposition of two single contraction curves.**—(F. p. 47, H. p. 274). For the purpose of observing this it is very advantageous to substitute the "pendulum myograph" for the revolving cylinder. The pendulum is provided with two trigger keys of the same kind as that employed in the last experiment, which can be so adjusted that they are opened by the pendulum in its swing. According to the distance at which they are placed, two circuits in which they are severally introduced, are broken at any desired time after each other. Connect two Daniells with two induction coils. Arrange the two trigger keys of the myograph, each in one of the two primary circuits. Connect the two secondary coils by one terminal of each; connect the remaining two poles with electrodes to the muscle, which must be fixed on the myograph plate in the same manner as in (2).

Make two experiments, one in which the second excitation follows the first at an interval of time shorter than one-hundredth of a second, the other in which the interval is prolonged to five-hundredths. In the second there is accumulation of effect, but not in the first.

29. **Composition of Tetanus.**—Use a revolving cylinder which rotates once in about ten seconds. The arrangement of the circuit must be as in 4. A key is required in the secondary circuit (See 13) and an interrupter of the following kind into the primary. (*a*) Fix one end of a steel spring connected with one wire in a clamp so that when it is made to oscillate the other end dips by its bent down point into and out of mercury contained in a cup to which the other wire is attached. Set the secondary coil at such a distance that the break shock is alone effectual. Make several experiments, first with the spring of such length that the interruptions occur three or four times in a second; a second with seven or eight interruptions per second, and so on until the spasms which were in the first experiment distinct become completely fused. (*b*) Substitute for the spring and mercury pool an interrupter which can be rapidly worked by the finger used as in striking a pianoforte key. Strike as frequently and regularly as possible—say seven times per second. If the muscle is fresh fusion will be incomplete. Faradise it once or several times and repeat the observation.

30. **Influence of fatigue.** (F p. 88). (*a*) Arrange an experiment exactly as in 27, but instead of closing the primary circuit for a moment only (see last line of paragraph) allow it to remain closed. A series of contractions are recorded. The height of the contractions at first increases, afterwards diminishes; the interval of time between the moment of excitation and that at which the lever attains its greatest height gradually increases. For the sake of distinctness record only one in ten of the curves, for which purpose the adjusting screw must be used to withdraw the lever from the paper during the intervening contractions. (*b*) Repeat the same experiment using a recording surface of which the

rate of motion is not more than a millim. per second. In this case each contraction is recorded. It is seen that during the observations the rate of diminution of effect is uniform, so that the line connecting the apices of the contraction curve is straight.

31. **Experiments relating to Electrotonus.** (F. p. 40, H. p. 337). In these experiments as in 5 *b*. the slowly revolving cylinder must be used. The muscle and nerve must be prepared as follows:—Strip off the skin, cut across the trunk half way down the back. Remove the viscera and the wall of the visceral cavity. Lay the preparation on the cork plate, ventral surface downwards. Expose the sciatic nerve in the hollow of the knee. Thrust one blade of the scissors between nerve and femur with its back to the nerve and divide the femur about a third of an inch from the knee. Free the nerve from surrounding parts following it to its origin, carefully severing its muscular branches. Cut away everything excepting the remainder of the vertical column to which the nerve is still attached, and lastly fix the cut-off end of the femur in the clamp of the myograph. In all cases in which as in the study of electrotonus, the nerve must be separated from the surrounding parts, it is necessary either to support and enclose it during the period of observation in a covered trough, or to place the myograph and preparation in a moist chamber, of such construction that the lever on which the muscle acts is not enclosed.

The muscle chamber (F. p. 72) contains two pairs of non-polarisable electrodes, the wires from which are connected with binding screws outside. To these the following form is given for economy of space. Each wire is soldered to a zinc rod, one end of which is carefully polished and amalgamated. Over the amalgamated end is drawn a sheath (like the finger of a

glove) of wash leather steeped in zinc sulphate solution. This is further enclosed in a wider sheath steeped in 0·6 per cent. solution of chloride of sodium, the outside of which is smeared with kaolin paste made with the same solution. The nerve is supported by a vulcanite trough which is supported horizontally by the pillar of the myograph in such a position as to receive the whole of the nerve conveniently when the preparation is in position. The sides of the trough have notches at intervals, through which thick ligature threads pass underneath the nerve; the opposite ends of each thread are tied together below. Before putting the threads in their places they are well soaked in salt solution and smeared with soft kaolin paste. The ends of the threads serve to bring them into connection with the sheaths of the zinc rods, which are supported by their wires in a suitable position for the purpose. Non-polarisable electrodes of this form can only be used in the moist chamber.

Use two Daniells for the "polarising" current. Connect them with the middle binding screws of a Pohl's reverser, from which two other wires proceed to the end blocks of the Rheochord (I. 16). Connect block A of the Rheochord with one of the electrodes *pp'* and the slider with the other. Connect the secondary coil of the induction apparatus with the electrodes *xx'*. Either single induction shocks or faradisation may be used to test the excitability of the nerve. If the latter, arrange the induction apparatus as in I. 8.

(a) Find the minimum distance of coil at which contraction occurs in the absence of the polarising current, and then remove the coil just beyond that minimum. Make the polarising current descending. If you are examining by single shocks, you see at each excitation strong single twitches; if by faradisation you see that the muscle enters into tetanus.

It has thus been shown that the excitability is increased in the vicinity of the cathode during the passage of the continuous current. *(b)* Push the coil a little within the minimum, so as to obtain evident contractions, or tetanus, according to your arrangement. Make the polarising current ascending. If you continue to test by single shocks, you find that they no longer cause contraction; if by faradisation, that the tetanus is cut short. The excitability is therefore diminished in the vicinity of the anode during the passage of the continuous current. *(c)* Employ single shocks to examine the after effects, viz., the state of excitability after the passage of the continuous current. You find that the excitability is increased after the passage of the continuous current in either direction.

32. **Demonstration of the Law of Contraction.** (F. p. 75, H. p. 338). For this purpose the arrangements required are the same as for experiments on electrotonus, with the exception that the induction apparatus, and the battery and electrodes connected with it, are not wanted. The key in the primary circuit must be a mercurial one, and in perfect order. Having completed the connections, close and open this key at intervals of two seconds, so as to make and break the primary circuit, gradually increasing the distance of the slider of the rheochord from the block until the muscle begins to contract at make. This effect is usually observed sooner, *i.e.*, with a weaker current when its direction is from the muscle. At once shift the slider to the second length. The muscle will probably respond to both make and break, whether it is directed from or towards the muscle. Now substitute for the two Daniells half a dozen Groves, or Lechanchés, and dispense with the rheochord. Under these conditions contraction occurs at make only when the current is towards the muscle, at break only when it is towards the spinal cord.

33. **Ritter's Tetanus.** (F. p. 76, H. p. 339). Conduct a continuous current of such strength as to give the third stage, through a nerve for a short time, the direction of which should be towards the spinal cord. On breaking the current, the muscle enters into tetanus, which can be instantly arrested by again closing the current, or by cutting the nerve near the muscle, but is not abolished by cutting the nerve midway between the electrodes. It is sometimes possible to observe the same effect on opening a current directed towards the muscle. If so, it can be abolished by severing the nerves between the electrodes. It is therefore dependent on conditions which have their seat at the anode.

34. **Experiments to Demonstrate the Seat of the Physiological Influence of Fatigue.**—Arrange two nerve-muscle preparations, A and B, loaded equally with 50 grms. each, with the nerves on the same pair of electrodes, connected with the secondary coil. Below this point, on the nerve B, apply the electrodes (unpolarisable) of a single Daniell, preferably so that the current is descending. Faradise, and at the same time make the continuous current; the muscle A, will enter into tetanus, B will remain quiescent, the stimulus being blocked by the electrotonic zone. Continue faradisation until the tetanus of A has quite subsided, then break the constant current; B will forthwith enter into tetanus. Both nerves have been equally stimulated, and are, therefore, equally fatigued; the tetanus of B shows that the excitability of its nerve was not exhausted, and consequently that the apparent exhaustion of A was not nervous.

Connect a nerve-muscle preparation with two pairs of electrodes, one pair to the nerve, the other to the muscle. Connect their wires to the two sides of a switch, so that you can rapidly transfer the current from one pair to the other.

Faradise the nerve until tetanus has quite subsided, then transfer the current to the muscle. The apparently exhausted muscle enters into tetanus. For both experiments the slowly revolving cylinder should be used.

35. Measurement of the **Period of Latent Stimulation** by the **Pendulum Myograph.** (F. p. 43, H. p. 271). Preparation of the apparatus. Cover the glass plate smoothly with paper, smoke its surface as before, and fix it to the pendulum. Arrange the "detent" and the "catch" so that the pendulum, when detached from the former, just catches on the latter. Test the instrument by taking tracings with a tuning-fork, vibrating 100 times a second, on the smoked paper, when the pendulum is moving at several different velocities (the velocity varying with the positions of the detent and catch). Arrange the electrical apparatus for single shocks, as in 4, including in the primary circuit one of the keys of the myograph. Prepare the gastrocnemius as in 27. Great care must be taken in fixing the femur immovably to the cork plate, in attaching the ligature (for which thin wire may be advantageously substituted) to the tendon and lever. See also that no part of the apparatus touches the surface of the glass plate, as the pendulum swings, excepting the writing point, and that the pressure of the point on the plate is slightly greater towards the end than at the beginning of the swing. Bring back the pendulum to its place and see that everything is in order—the keys closed, the lever in its position, the electrodes under the nerve, etc. On liberating the pendulum, a muscle curve is inscribed on the smoked surface. Withdraw the lever from its writing position, bring the pendulum back past the key, close the latter, keeping it closed by firm pressure of the finger, allow the pendulum to rest against it, bring the lever into the writ-

ing position, and make a mark on the surface, which indicates the moment of excitation. Take three or four similar curves, depressing the table an equal distance after each observation ($\frac{1}{4}$ or $\frac{1}{2}$ turn) by the handle. Remove the muscle lever, and take a tracing with a tuning-fork, vibrating 100 times a second, carefully arranging the style of the fork in the position previously occupied by the writing end of the muscle lever. Remove the paper, varnish and measure the tracings. From the mean result of the measurements, the latent stimulation may be computed.

36. **Rate of Propagation in Nerve.** (F. p. 45, H. p. 345). *(a)* In the frog.—The arrangements are the same as for the last experiment. The nerve must be placed in a vulcanite trough similar to that employed in 31, with the exception that instead of threads, two pairs of wires cross the floor of the trough at a distance of about an inch from each other. The nerve must be prepared with great care, as in I., 3, and must be in contact with both pairs of wires. Connect each couple of wires with the side binding screws of a Pohl's reverser from which the cross wires have been removed, and the wires of the secondary coil to the middle screws, so that by turning over the bridge, the near and the distant portion of the nerve can be excited alternately. Cover the trough with a flap of muscle, taking care that the flap does not touch the nerve. Make a series of observations, throwing over the bridge between each. Then take a tuning-fork tracing, varnish, measure the length of nerve between the two contacts, and calculate therefrom the rate of propagation. *(b)* The pendulum myograph may also be used to measure the rate of propagation in human motor nerve, the observer experimenting on himself. Arrange a Marey's tympanum, so that its lever may write on the glass plate. Connect the tympanum

by an elastic tube in which there must be a small aperture for the escape of air, with a pair of toy bellows held between the thumb and finger of the left hand. Arrange the primary circuit as before. Connect one electrode of large area from the secondary coil with any part of the body. Apply a small metal disc covered with wash leather steeped in strong solution of salt to the skin, first at the bend of the elbow, and for a second observation, above the clavicle, arranging the lever of the tympanum so that the two curves shall be close together. Then make a series of similar observations in pairs, taking care to allow the pendulum to draw a base line to each curve. Measure on the base line the distance between the curves of each pair, rejecting all records in which the two are not of the same amplitude. The results obtained by this rough method are surprisingly constant. They show that the rate of propagation in man much exceeds that observed in the frog.

37. **The Elastic Properties of Muscle.**—*(a)* For experiments on this subject, use the slowly revolving cylinder, and a counterpoised writing lever, two or three feet in length. Arrange the gastrocnemius as in 31, substituting a scale pan for the weight. Attach the tendon and scale pan to the lever at as short a distance as possible from the writing point. Prepare four equal weights of from 20 to 25 grammes each, and cautiously place them in succession on the scale pan while the cylinder is revolving. Observe that the extension is greater for the first weight, less for the next, and so on; and that the increase of length after each addition of weight is gradual. On removing the weight, the muscle resumes nearly its original length, which, however, it never completely attains, although it continues to shorten for some time after it ceases to be acted upon by the weight.

(b) To compare the extensibility of the muscle during rest and action, load it successively with two weights, say of 50 grammes and 10 grammes. Determine the height to which the point of the lever rises in tetanus with the two weights in succession, and compare the difference between the two, with the extension of the untetanised muscle, when successively loaded with the same weights.

II.—The Frog Heart.

1. **Rhythmical Motions.**—In a curarized preparation of which the hemispheres have been destroyed, expose the sternum, and cut across the episternal cartilage. Then sever the sternum from its connections by a cut on either side, and turn it down over the belly. The heart is seen still covered by the pericardium. Note the condition of each of its cavities, and the mode of its rhythmical action.

2. **The Inhibitory Centre.** (F. p. 170). For the purpose of observing the effect of passing series of induction shocks through the inhibitory centre of the heart, a fine ligature is attached to the frænum (the thread-like ligament which stretches from the dorsal aspect of the ventricle towards the lower part of the pericardium). By means of the ligature, the heart is raised out of its place and turned upwards. The inhibitory centre is recognized by the whitish, crescent-shaped line, which marks the junction of the wall of the sinus with that of the right auricle. Faradise this spot for a second, or less, placing the points of the electrodes on the line, a couple of millims. distant from each other. Observe the mode and order in which the cavities of the heart resume their rhythmical action.

3. Destroy the spinal cord by pithing, and observe the changes thereby produced in the state of the circulation, and particularly in the mode of action of the heart.

4. **The Cardiac Vagus of the Frog.**—*(a)* Preliminary Dissection.—Expose the trunk of the vagus nerve as it escapes from the cranium as follows:—Remove the integument so as to bring into view the muscles of the back of the neck on one side, avoiding injury to the cutaneous vessels. Then expose the scapula, and sever with the scissors the cartilaginous from the bony scapula; remove the former, dividing the muscles attached to it, then expose the sterno-mastoid muscle which connects the outer part of the petrous bone and the posterior border of the cartilaginous ring of the membrana tympani with the concave anterior border of the scapula. Remove or draw aside the sterno-mastoid so as to expose the slender muscles (petrohyoidei) which run from the petrous bone to the posterior horn of the hyoid bone, embracing the cavity of the pharynx. Parallel with these muscles, and in close relation with them, are seen the carotid artery and several nerves, of which the two nearest the cranium are the glosso-pharyngeal, and the vagus.

(b) Expose the vagus in a pithed preparation. Expose the heart, as in 1, and introduce a small test tube into the gullett. Fix the preparation in such a position on a cork, that the electrodes can be conveniently applied to the nerve, at the same time that the motion of the heart can be observed.

5. **The Stannius' Heart.**—Prepare a frog heart with frænum ligature as before. Then pass a thick ligature under the bifurcation of the aorta between it and the venæ cavæ superiores. Then, seizing the frænum ligature with the forceps, turn the heart up. Carefully observe the position of the

"crescent," and loop the ends of the ligature so that when it is tightened it may embrace the crescent. On tightening, the heart will stop in diastole.

In the heart so prepared, sever the ligatured parts from the rest of the preparation with sharp scissors. The auricles and ventricles resume their normal rhythmical action.

Cut off in a preparation which has been so treated, the remainder of the auricles and the bulb, leaving the ventricle and auriculo-ventricular septum. The heart continues to beat normally, or, if the beats cease, they are renewed by a pinch, by an induction shock, or by bringing a hot wire into the neighbourhood of the cut surface.

6. **Localization of the Motor Centres.**—In one of two such preparations (called ventricle preparations) which beat rhythmically, cut off the whole of the auriculo-ventricular furrow with sharp scissors. The preparation so obtained (the ventricle apex) does not contract spontaneously, but *responds* to a single excitation, whether mechanical or electrical, *by a single contraction*, the duration of which is dependent on the temperature. In the other preparation, divide the ventricle by two parallel cuts into a middle and two lateral thirds. The middle third includes the ventricular border of the inter-auricular septum, the right lateral third contains the root of the bulb. The middle third beats rhythmically, the lateral thirds respond to excitations by single contractions, but do not beat of themselves.

7. **Action of Muscarin and Atropin.**—In an entire heart (a heart removed by severing the vessels, for which purpose the organ should be lifted out of the pericardium by a ligature tied to the frænum), stop rhythmical action by applying to it a drop of serum containing a trace of muscarin. Observe the relaxed and motionless condition of the ventricle.

After a few minutes, apply (in serum) a drop of 0·2 per cent. solution of atropin. Observe the gradual restoration of rhythmical action in the atropinized heart. Observe that faradisation of the inhibitory centre is without effect.

8. **Action of the Constant Current on the Contractile Substance of the Heart.**—For this purpose prepare electrodes as directed in 1. Fix a cork vertically on a sheet of lead about an inch and a half square; cover the top of the cork with wax mass, the upper surface of which should be somewhat concave. Place the support on a sheet of wet filtering paper and cover it with a beaker. Attach a fine ligature to the frænum, and remove the heart after severing the principal vessels. Collect some blood and dilute it with as much as 0·75 per cent. salt solution, and place a few drops of it on the wax surface.

Make a "ventricle-apex preparation," as directed in 6. Having ascertained that it does not beat rhythmically of itself, fix it in its place by the aid of fine glass pins and replace the beaker.

Prepare and arrange two Grove's cells in circuit, interpose a key and a pair of electrodes. Fix the electrodes, so that their points are in contact with the apex and base respectively of the preparation. The passage through the ventricle apex of a voltaic current in the direction of its axis, produces rhythmical action, which lasts as long as the current passes.

9. **Study of the Ventricular Systole by the Graphic Method.**—Prepare a writing lever consisting of a glass rod about $\frac{1}{40}$ inch in thickness, and five inches long, having at one end a knob of glass, and at the other a writing point. This is thrust through a square bit of cork, which is then pushed up to the knob. A fine steel needle passes through the cork at right angles to the rod. The rod also bears,

close to the needle, a vertical arm of cork, by means of which it rests on the ventricle. The preparation lies on a metal plate, which forms the upper end of a cylindrical brass box, through which water, at any desired temperature, can be passed. This plate is furnished with bearings in which the steel axis of the lever works. The metal box is fixed to one of the adjustable supports of the recording apparatus.

(a) The rhythmically contracting heart.

Expose the heart as before. Raise it from the pericardium by a ligature attached to the severed frænum, and cut through the vessels. Place the heart on a plate, adding a few drops of dilute serum, and arrange the lever so that the cork arm rests on the ventricle, and the writing end inscribes its movements on the blackened surface of the cylinder. The rate of motion should be about 20 inches per minute.

Allow water at 12° C. to pass through the cylindrical box and record the rhythmical contractions of the ventricle. Repeat the experiment, substituting water at 17° and at 22°, and compare the tracings.

(b) The curve of a single ventricular contraction.

Prepare finely pointed electrodes, as in I. 1, arranging for single induction shocks. Fix the electrodes to an adjustable support, so that they can be brought with precision into contact with the preparation. Prepare a Stannius' heart and arrange it for recording as in *a*. Adjust the electrodes, taking care not to interfere with the lever. Place the secondary coil at about 10 centimeters distance from the primary, or nearer, if on trial it is found necessary to do so. Then bring the point of the lever into contact with the blackened paper, so as to write a base line or abscissa, and open the key. The rate of motion of the recording surface should be about 2½ inches per second.

In order to obtain series of tracings which can be conveniently compared, introduce into the primary circuit the self-acting key described in I., 28. In this way a number of curves may be drawn on the same abscissa, or on parallel abscissæ at convenient distances from each other. Having practised one or other of these methods, proceed to make the following observations:—

α. When a succession of ventricular curves are drawn at temperatures varying from 12° to 18°, C., it is found that the duration of the systole is increased by about 0″·1 for every degree of temperature.

β. When the ventricle is excited by single induction shocks, following each other at about 10″ intervals, each curve is observed to exceed its predecessor in amplitude, the augments gradually diminishing from the beginning to the end of the series.

γ. In the muscular tissue of the heart, the period of latent stimulation is much longer than in voluntary muscle. Its duration is about 0″·15. To measure it, a vertical line must be drawn on the recording surface, indicating the position of the writing point at the moment that the trigger of the cylinder comes into contact with the lever of the self-acting key. (*See* I., 28).

III.—Functions of the Spinal and other Reflex Centres of the Frog.

1. (F. 537, H. 479). The preparation to be used in the following experiments is obtained by severing the spinal cord immediately behind the medulla oblongata, and introducing, by the opening made for this purpose, a wooden plug into the cranial cavity, so as to destroy its contents. This having

been done, it is placed on a sheet of moist filter-paper, resting on its ventral surface with the hind limbs extended, and covered with a bell jar. For a time it remains motionless, but eventually assumes a position which differs but little from that of a living frog. Observe the differences.

2. Prepare half-a-dozen pieces of filter-paper, each an eighth of an inch square, and some strong acetic acid. Turn the preparation over, and after observing that the natural position is not resumed, apply one of the squares, after moistening it with acetic acid and drawing off excess by touching with dry filter paper, to the inside of the right thigh, and observe the result. Repeat the experiment, holding the right foot. Next, attach the preparation to a suitable holder in such a way that the trunk may be steadily supported and the limbs may hang freely, and apply the squares in succession to different parts of the surface, as *e.g.*, to the skin on either side of the tendo Achillis, or to either flank. Observe in each case that the muscular response which results from excitation of the same part of the surface of the body is always the same.

3. Arrange a second preparation as last described, using a holder so constructed that the limbs may be suspended at any desired height above the table. Prepare several beakers of water acidulated respectively with 1, 2, 3, 4, and 5 per thousand of sulphuric acid, and place some of each mixture in a saucer. Beginning with the weakest of the acid liquids, bring down the preparation with the rack and pinion, until the tip of the longest toe is immersed. Repeat the experiment at intervals of three minutes with the stronger liquids, in order, carefully washing the foot after each excitation, by dipping it into a beaker of water. Measure the time which intervenes between the beginning of the excitation and the muscular response in each case, with the aid of a metronome.

4. Observe carefully the attitude of a brainless frog when left to itself, and its behaviour when placed on its back, on an inclined surface, or in water, as well as when excited by cutaneous stimuli, comparing the phenomena observed with those which exhibit themselves in the spinal cord preparation.

5. Proceed as in 1, substituting a preparation in which, after destruction of the brain, a couple of drops of a 0·1 per cent. solution of sulphate of strychnia have been injected under the skin of the back. Observe that instead of co-ordinate muscular responses, cutaneous excitation produces, under the influence of strychnia, paroxysms of convulsion, in which the body and limbs assume a characteristic attitude.

IV.—Sensation and Perception.

1. **Time Occupied in the Simplest Mental Processes.** (F. 594, H. 511). To measure the time required for responding to a signal (reaction time or personal time), the simplest plan is to arrange a battery circuit in such a way that it is closed by the same act by which the observer makes the signal, and that it is opened by the response of the observed person. Whatever be the nature of the signal, the requirements are:—(1) Two Grove's cells arranged in circuit; (2) a break key, (a lever resembling in shape a pianoforte key, which, when touched, breaks a mercurial contact); (3) a du Bois' key; (4) an electro-magnet with a light lever attached to its armature; (5) a chronograph; (6) a recording surface, of which the rate of motion is not less than 1 foot per second. The battery, two keys, electro-magnet, and chronograph, are arranged in circuit, and in such positions that the electro-magnet lever may be in the neighbourhood of the observed person, and the

du Bois' key, cylinder, and chronograph, in reach of the observer. On closing the circuit, the lever is drawn towards the magnet and gives the signal. The signal may be an induction shock through the tip of the tongue, (in which case an induction coil must be in circuit in addition to the instruments above mentioned), a touch on the hand given by the lever, a sound, or a visible signal, such as a white disk, letter, or number, suddenly brought into view.

2. **Tactile and Muscular Sensation.** (F. 529, 530, H. 463-469). In all the following experiments two persons must take part, one of whom must vary the conditions without the knowledge of the other, and note the results. In the experiments relating to the sensations of pressure, locality, and muscular exertion, the observed person must have his eyes shut.

The appreciation of **Temperature** must be tested by immersing the same surface successively in water of slightly different temperatures. The smallest differences can be detected when the temperatures of the liquids compared, approximate 30° C.

To test the sensation of **Pressure**, the hand or other part to be investigated must be entirely at rest, and supported on a horizontal surface. The weights used must be moderate—from a pound to four or five pounds; in which case it will be found that a difference between two weights of one-thirtieth can be detected.

For testing the sensation of **locality** in any part of the surface of the body, a pair of compasses is used, of which the points are provided with cork sheaths, having smooth blunt ends. The points being at first at such a distance that when both touch the skin or mucous membrane of the tongue, they are distinctly felt as two, they are gradually brought nearer

until the two impressions blend into one. The smaller the distance at which this happens, the finer is the sensation of locality in the region investigated. Another method is that of interrogation. The observer touches the skin, and asks the observed person to designate the locality touched.

The sensation of **muscular exertion** is tested by experiments, each of which consists in lifting in succession, two weights, of which one is heavier than the other by a small but perceptible difference; this difference is diminished at each trial until it can no longer be appreciated. As it is essential that sensation of pressure should be excluded, the weight to be estimated must in each trial be enclosed in a handkerchief, of which the corners must be held in the hand.

For the investigation of the sensation of **taste** and of the limits of the gustatory region, four test liquids should be prepared, viz., saturated solution of sulphate of quinine, 10 per cent. solution of common salt, 3 per cent. solution of sugar, and 0·1 per cent. solution of citric acid. These liquids represent the four fundamental sensations, each of which may be tested separately, or two alternately. In each experiment a camel-hair pencil is dipped in the liquid, drained by touching it with filter paper, and applied for a moment to the surface. To secure freedom from bias on the part of the observed person, trials should be made in which tasteless liquids, or liquids of different tastes are alternated in various orders, care being taken to irrigate the surface between each trial and the following one, with water.

The voltaic sensations of taste are experienced when two zinc plates, which form the terminals of a Grove's element, are applied respectively to the upper and under surface of the tongue as far back as possible. As the effect differs according to the direction of the current, a reversing key must be introduced into the circuit.

Part II.

DEMONSTRATIONS.

I.—Mode of Measuring and Recording the Arterial Pressure.—Use of Recording Apparatus.

The instrument used is called a kymograph. The arterial cannula is a T-shaped tube of glass. By its stem, it is connected with the manometer (a U-shaped glass tube containing mercury). One branch of the T is drawn out and bevelled so as to be easily introduced into the artery: to the other is fitted a short piece of india-rubber tubing, guarded by a steel clip. The stem of the cannula communicates with the proximal arm of the manometer by an unyielding tube of lead or gutta-percha. The proximal arm (that connected with the cannula) also communicates by a long flexible tube with a bottle containing solution of bicarbonate of sodium under pressure. The manometer is fixed to the recording apparatus, so that its oscillations are inscribed on the moving surface. This is effected by means of a style carried by a vulcanite rod, which floats on the surface of the mercury in the distal (open) limb of the manometer. The recording cylinder is driven by clockwork; it is either covered with smoked glazed paper, or is fed by an endless roll of paper, in which case, a sable pencil, charged with coloured ink, is substituted for the style. The paper surface in either case moves at a uniform rate of 20 inches per minute.

The artery used is the carotid of the rabbit. The distal end of the prepared part of the vessel is ligatured. The

proximal end is temporarily closed by a spring-clip. The vessel having been opened near the ligature, the cannula is introduced and secured in its place by a second ligature, its drawn-out end being directed towards the heart. This done, the guttapercha tube of the manometer is connected with the stem of the cannula, and the whole system filled with solution of sodic bicarbonate under a pressure of about four inches of mercury. On removing the clip on the artery, communication is established between the arterial system and the manometer, which now records the variations of arterial pressure. The tracing exhibits larger (respiratory) undulations, on each of which many smaller undulations (cardiac pulsations) are inscribed. It shows (1) that each contraction of the left ventricle produces a momentary increase of arterial pressure; (2) that the pressure increases after each inspiration, and sinks in the interval; (3) that during the rise of pressure the pulsations are more frequent than during the fall. **Excitation of the Cardiac end of the divided Vagus** by faradisation, produces (if weak induction currents are used) diminution of the frequency of the heart's pulsation and of the arterial pressure. If stronger currents are used, the heart is arrested in diastole.

[N.B.—In each of the Demonstrations, I., II., and III., a rabbit is used, which is rendered completely insensible by a suitable anæsthetic, and is killed before recovery.]

II.—The Normal Respiratory Movements. Influence of the Vagus Nerve, and of its Centre. Apnœa and Dyspnœa.

The motions of a metal plate which is kept in constant contact with the posterior surface of the central tendon of the

diaphragm of the rabbit, by the pressure of a spring, are communicated by a long steel wire to the vertical arm of a bell-crank lever. The horizontal arm of the lever is prolonged, and bears a style by which an enlarged record of the respiratory motion of the diaphragm is inscribed on the cylinder of the recording apparatus. The rate of movement of the cylinder is the same as in the last demonstration.

The inspiratory contraction of the diaphragm is expressed by the descent of the writing style, its relaxation by the ascent, which is at first rapid, but afterwards more gradual.

Apnœa.—When by excessive artificial respiration the circulating blood becomes overcharged with oxygen, all respiratory movement ceases. On discontinuing the injections of air, the respirations after a time begin again; at first they are scarcely perceptible, but each exceeds its predecessor in extent, until the normal is reached.

Dyspnœa.—When an atmosphere containing an inadequate percentage of oxygen is respired, the opposite effect to that described above is produced. The respirations become more ample and more frequent, and the auxiliary muscles are brought into action. No such effect is produced by an atmosphere containing as much as ten per cent. of CO_2, provided that the supply of oxygen is sufficient.

Excitation of the Superior Laryngeal Nerve.—Excitation of the central end of the trunk of the superior laryngeal nerve by faradisation, arrests the respiratory movements, the diaphragm becoming stationary in the position of expiration. When extremely feeble currents are used, rhythmical movements may continue at long intervals. Introduction of irritant gases or vapours into the larynx produces similar effects.

Similar excitation of the central end of the divided vagus, below the cricoid cartilage, produces effects which differ ac-

cording to the strength of the induction currents employed. When currents of moderate strength are used, the diaphragm remains, during the excitation, in the position of inspiration, the state of contraction being, however, usually interrupted by momentary relaxations at short intervals.

III.—Functions of Vascular Nerves.

Constricting Nerves.—Division of the trunk of the sympathetic opposite the cricoid cartilage, is followed by dilatation of the central artery of the lobe of the ear on the same side, and increase of vascularity. On comparing the temperature of the congested lobe with that of the other side, it is found to be two or three degrees higher. The pupil of the same side is more contracted than the opposite one. Excitation of the end next the superior ganglion produces constriction of the central artery, and abolishes the congestion of the lobe.

Dilating Nerves.—Excitation of the central end of the great auricular nerve (or of the posterior auricular) produces temporary vascular changes, which are identical with those permanently produced by section of the sympathetic.

Depressor Nerve.—Excitation of the central end of the divided depressor occasions general diminution of arterial pressure (dependent on dilatation of the blood-vessels supplied by the splanchnic nerves). If the vagi have been previously divided, the diminution of pressure is not associated with any change in the frequency of the contractions of the heart.

IV.—Movements of Circulation and Respiration in Man.

1. **The Cardiograph and Sphygmograph.**—*(a)* Two receiving tympana (cardiographs) are used. One is applied to the seat of the cardiac impulse, the other to the carotid artery. The two recording tympana with which these are severally connected, inscribe the motion of the heart and that of the artery respectively, on the same cylinder. The arterial expansion follows that of the heart at an interval of about eight-hundredths of a second. The duration of the ventricular impulse is about three-tenths of a second.

(b) The sphygmograph having been adjusted so as to record the radial pulse, a receiving tympanum on the carotid is connected with a recording tympanum attached to the frame of the sphygmograph, so that its lever writes on the same surface as that of the sphygmograph. The interval of time between the impulse of the carotid and that of the radial is about the same as that between the carotid and the heart.

2. **The Stethograph.**—The changes of form of the thorax in respiration are investigated by the measurement of the diameters of the chest. The most important diameters are, the antero-posterior (from upper end of sternum to third dorsal spine, 150 millims., and from lower end of sternum to eighth spine, 200 millims.); the transverse (at the eighth rib, about 230 millims.). These measurements refer to an adult male, as taken during the respiratory pause. The first of these diameters increases about a millimeter, the second about two millimeters, and the third about two and a half in ordinary tranquil inspiration. These measurements, when recorded by the stethograph, yield the "respiratory curve."

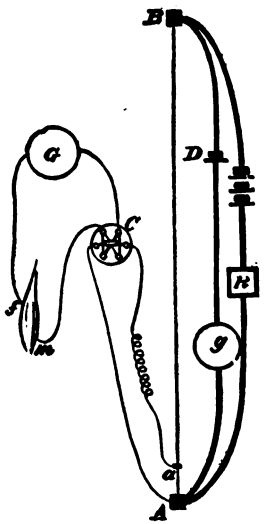
V.—Electromotive Phenomena of Muscle.

The most important instrument used is a Thomson's Reflecting Galvanometer, of high resistance, the terminals of which are connected by insulated copper wires with non-polarisable electrodes. These are in contact by their clay plugs with the two surfaces to be compared.

To the needle of the galvanometer a light concave mirror is attached, on which a beam of light falls and is focussed, after reflection, on a divided screen. Thus the smallest deflection of the needle (by which any electrical difference between the two contacts is indicated) can be exactly measured. By means of a suitable shunt, either the whole, a tenth, or other decimal fraction of any current flowing through the circuit can be led through the galvanometer.

The scale-reading of the galvanometer is proportionate to the current passing through it, but affords no indication of the difference of potential between the two surfaces compared. For this purpose it is necessary to balance the current in the galvanometer circuit due to the electromotive force of the muscle, by an opposed current of which the electromotive force is known. The instrument used for this purpose is called a **Compensator.** (*See* fig. 4). Two blocks, *A* and *B*, are connected by a wire. They are also in connection (1) with the two poles of a Standard Battery (*D*) by wires, one of which passes through a multiplier, and (2) by two other wires, one of which passes through a rheostat, with the corresponding poles of a battery of several cells. This must be of such strength that when the resistance of the rheostat is made as small as possible, the battery, *D*, is over compensated. The resistance is then increased until the galvanometer *(g)*

FIG. 4.



is at zero. Under these conditions the difference of potential between *A* and *B* is equal to the electromotive force of the standard cell. If, therefore, as shown in the diagram, a part of the rheochord wire, *Aa*, included in the same circuit with the galvanometer *G*, is of such length that the current due to the electromotive force of the muscle, is exactly balanced, so that there is no deflection, the difference of potential between the two led off surfaces *f*, and *m*, is to the e.m. f. of the standard cell, as the distance, *Aa*, is to the whole length of the wire.

All of the electromotive properties of muscle may be demonstrated more advantageously and strikingly with the aid of the capillary electrometer, than by the galvanometer, first because it indicates differences of potential, irrespectively of resistance, and secondly because transitory changes in the electrical relations of two "led off" surfaces (such as those

which accompany the excitatory process in muscle) can be observed by it.

1. **Electromotive Phenomena of Muscle.**—The gastrocnemius muscle of the frog is used. One of the electrodes is in contact with the convex surface of the muscle near its upper end, the other with the expansion of the tendo Achillis. In this arrangement the surface of the tendon is negative to that of the muscle.

2. On exciting the muscle by faradising its nerve, a deflection takes place in such a direction as to indicate that the electrical difference between the two surfaces is diminished. After excitation the needle resumes its former position.

If the capillary electrometer is used, it is seen that the mercury column oscillates during the period of diminution, and that the number of oscillations per second corresponds to the number of excitations to which the muscle is subjected in the same time.

3. The electrode in contact with the tendinous expansion is now brought near to its fellow, so that both contacts are now muscular. They are nearly isoelectrical. On injuring the lower of the two contacts mechanically, or by heat, it becomes at once strongly negative. On excitation of the nerve by induced currents, the negativity diminishes as before.

4. **Electromotive Phenomena of the Ventricle of the Frog Heart.**—A Stannius' heart preparation (*See* Part II., 5) is "led off" by contacts at its apex and base. If the heart is uninjured, these surfaces will be found to be nearly isoelectrical. On injuring either surface it becomes negative.

2. A normally contracting heart is led off by contacts similarly situated. Each contraction is accompanied by a deflection of the needle, indicating that the apex becomes first

positive then *negative*. By injuring the apex, mechanically or otherwise, the deflection becomes entirely positive.

3. A ventricle preparation (Part II., 6) is led off at apex and cut surface. During contraction, the effect is similar, but the negative deflection is much larger.

4. A ventricle apex preparation (which does not contract spontaneously) is led off as above. Its cut surface is at first strongly negative to the apex. On excitation at the base by a single induction shock, the ventricle contracts, its contraction being accompanied by a deflection indicating that the apex becomes *negative*.

Part III.

ELEMENTARY EXERCISES IN CHEMICAL PHYSIOLOGY.

I.—Starch, Dextrin, Dextrose, Fat.

1. **Starch** is insoluble in cold water.

2. It dissolves imperfectly in hot water; the liquid so obtained is opalescent.

3. It gives a blue colour with iodine, which vanishes when the liquid is heated, but returns on cooling, if the heating has not been prolonged.

4. **Dextrin** is soluble in water.

5. The solution gives a red-brown colour with iodine, which vanishes on heating.

6. Commercial **Grape-sugar** is a yellowish-brown, crumbly substance, which is readily soluble in water. Its solution is usually slightly coloured, and reduces alkaline solutions of cupric hydrate.

7. The **Copper test.**—To a small quantity of ten per cent. solution of cupric sulphate, add about 5 c.c. of the liquid to be tested; then solution of caustic potash drop by drop until the solution is clear, and heat gradually. If dextrose is present, the blue colour vanishes, and a yellow precipitate appears of cuprous hydrate, or a red precipitate of cuprous oxide.

8. Conversion of starch into reducing sugar. Boil about 50 c.c. of starch solution in a flask with a drop of 25 per cent. sulphuric acid for five minutes. The liquid becomes limpid. It contains in addition to dextrose much soluble starch (**Amidulin**).

9. **Fat.**—Lard is insoluble in water. By boiling with potash it yields a solution of soap.

10. Decompose the solution by adding a few drops of dilute sulphuric acid. On heating, a layer of fatty acid collects on the surface.

11. **Microscopical Preparations.**—Starch grains; their disintegration by hot water; action of iodine on them. Crystalline forms of fatty acids.

II.—Milk, Flour, Bread.

1. **Milk** has (in London) usually an acid reaction, and a specific gravity of from 1025 to 1030. After removal of the cream, the specific gravity is higher.

2. Milk contains fat, sugar, and proteids.

a. **Proteids.**—Heat about 50 c.c. of milk to 40° C. in a flask. Add a few drops of rennet-extract, keeping the milk at the same temperature until a coagulation is formed.

b. Dilute 5 c.c. milk with eight or nine times as much water, acidulate with a drop or two of acetic acid, and warm as before. Strain off the coagulated casein through muslin.

c. When milk is filtered under pressure through a porous disk, its casein, being particulate, remains behind. The clear filtrate contains **lactose** (milk-sugar) and salts.

d. **Sugar.**—The strained liquid from *a* (**whey**) contains lactose, which, like dextrose, reduces metallic oxides. Apply the copper test (I., 7).

e. **Fat.**—The coagulated casein contains much fat (**butter**) which can be extracted by ether. The ether extract, when evaporated on paper, leaves a greasy stain.

3. **Flour.**—Wash about a desert-spoonful of sound flour in a muslin bag.

a. A milky liquid passes through containing much starch (I., 3) but no sugar.

b. After washing for some minutes, a sticky and tenacious material remains on the muslin, which can be collected; this, after further washing, forms an elastic mass (**gluten**) which can be drawn out into threads, and on burning, gives off the smell of burnt feathers characteristic of a proteid.

4. **Bread.**—Digest with warm water. The extract contains starch (I., 3) and dextrose (I., 7). The residue consists principally of starch and gluten.

III.—Albumin and its Acid and Alkaline Modifications.

1. **Albumin.**—White of egg (albumen) when diluted with water, strained and filtered, yields a faintly opalescent liquid. This liquid contains a proteid body, **albumin,** which diffuses through an animal membrane with great difficulty (V., 3).

2. Such a liquid, containing 5 per cent. of albumen, is to be used in the following experiments. It coagulates on heating at about 70° C. if neutral.

3. To some of the liquid add a few drops of 0·1 per cent. solution of caustic potash, and warm gently for two or three minutes. Boil. The liquid will no longer coagulate, the albumin having been transformed into the alkaline modification (**alkali-albumin**).

4. In a similar way treat another portion with a few drops of very dilute sulphuric acid (0·1 per cent.). Warm very gently for not less than five minutes. On boiling no coagulation occurs, the albumin having passed into its acid modification (**acid-albumin, syntonin.**)

5. Cool some of the liquid obtained in 3. Colour it with litmus solution, and add carefully very dilute acid.

A precipitate falls on neutralization, which is soluble in excess of acid.

6. Make a similar experiment with the liquid obtained in 4, substituting weak solution of potash for weak acid. A similar precipitate occurs on neutralization, which is soluble in excess.

7. Take three portions, of 5 c.c. each, of the original liquid in three test-tubes, and colour them with litmus. Dilute the 0·1 per cent. acid about 5 times, and add a drop of it to one of the portions; to another add a drop of potash solution similarly diluted. Heat all three tubes gradually, and note the temperature at which each coagulates.

8. Make alkali-albumin solution as in 3. Divide it into two equal parts. To one add two or three drops of 10 per cent. solution of sodic phosphate. Colour both with litmus, and neutralize with weak acid. The portion without sodic phosphate is precipitated. The other portion is not precipitated until enough acid has been added to convert the sodic phosphate present into acid sodic phosphate.

IV.—Characteristics of Proteids.—Peptic Digestion.

1. Tests for proteid bodies in solution.

a. To some of the albuminous liquid referred to in III., 2, add strong nitric acid. The precipitate obtained turns yellow on boiling.

b. Cool the liquid in *a* and add strong ammonia. The precipitate assumes an orange tint (**Xanthoprotein reaction**).

c. To another portion add **Millon's reagent.** (Mercury is dissolved in its own weight of strong nitric acid. The solution so obtained is diluted with twice its volume of water. The

decanted clear liquid is Millon's reagent). A precipitate is formed which turns dull red on boiling.

d. To a third portion add solution of potassic ferrocyanide, and a drop of acetic acid. A white precipitate appears.

e. Introduce a fourth portion of the liquid into a test-tube containing one drop of ten per cent. solution of cupric sulphate. On adding solution of potash, a violet colour is obtained (compare v., 2, *b*).

2. **Serum-globulin.**

a. Neutralize 5 c.c. of serum with a few drops of 0·1 per cent. sulphuric acid. Dilute with about 75 c.c. of water, and allow the precipitate to settle. The precipitate is insoluble in water, but soluble in excess of acid.

b. Dilute 5 c.c. of serum with 75 c.c. of water, and pass through it a stream of CO_2. The liquid becomes turbid as in *a*.

c. Repeat *b* without dilution. No precipitate is formed.

d. Add to a saturated solution of sulphate of magnesium a small quantity of serum. A copious precipitate is formed.

e. Pour over some fibrin contained in a watch-glass some solution of peroxide of hydrogen. Bubbles of oxygen are given off. If some tincture of guaicum be added, a blue colour is developed. Gluten, potato peelings, and many other substances develop a blue colour under the same conditions.

3. **Peptic Digestion.**

a. Introduce some fibrin into a test-tube, and just cover it with 0·2 per cent. solution of HCl. Allow it to stand for forty-five minutes in a water-bath at from 35° to 38° C. At the end of this time the fibrin is swollen and transparent, but has not dissolved.

b. Repeat *a*, using instead of hydrochloric acid, water to which a drop of glycerine extract of gastric mucous membrane has been added.

The fibrin remains unaltered.

c. Repeat *a*, adding a drop of the same extract to the acid liquid. The fibrin dissolves gradually.

d. Colour with litmus the liquid obtained in *c*. Neutralize carefully with weak solution of caustic potash (III., 6). The acid albumin formed during the first stage of digestion is precipitated.

V.—Pancreatic Digestion.—Amylolytic Ferments.—Glycogen.

1. **Pancreatic Digestion.**

a. Introduce 5 c.c. of one per cent. solution of sodium carbonate, to which a couple of drops of glycerine extract of pancreas have been added, into each of two test-tubes. Boil one of them and allow it to cool. Add some boiled fibrin to each, and place them both in the water-bath at 35° C. Compare the changes produced with those observed in peptic digestion (IV., 3, *c*).

b. Examine the liquid product of a pancreatic digestion, previously prepared by allowing a finely divided ox pancreas to digest itself in a 1 per cent. solution of sodium carbonate. It is alkaline, and may have a characteristic and offensive odour.

c. Boil some of this liquid after acidulating slightly. Albumin is coagulated.

d. Colour another portion with litmus, and neutralize carefully (III., 5); alkali-albumin is precipitated.

e. In a liquid obtained by concentrating the product above referred to, after having separated the greater part of the proteids contained in it, test for **Tyrosin** by adding Millon's

reagent, and boiling. The presence of Tyrosin is indicated by the reddish colour assumed by the liquid.

f. From such liquids **Leucin** usually separates on concentration, and can be recognized under the microscope by its crystalline form.

2. **Peptones.**—A solution obtained either by pancreatic or peptic digestion can be used.

a. The solution yields no precipitate either by boiling or by neutralization, but is precipitated by alcohol.

b. When concentrated and treated as in IV., 1, *e*, it gives a red instead of a violet colour.

The liquid product of the slow putrefaction of proteids resembles in most respects that of pancreatic digestion. To the latter, the presence of septic organisms is not essential.

c. Peptone, although more diffusible than other proteids, does not diffuse through parchment paper.

3. **Indiffusibility of Proteids.**—Suspend a parchment paper tube containing diluted blood, in a beaker of distilled water, so that the two open ends are above the surface. The colouring matter and proteids do not pass through the membrane. The soluble salts pass through readily, and their presence in the water can be recognized by the usual tests.

4. **Amylolytic Ferments.**—Prepare some starch solution and ascertain that it contains no dextrose (I., 2 and 8). To another portion add saliva, and place the tube containing the mixture in a water-bath at from 35° to 38° C. After a short time, the product will be found to contain dextrose.

5. **Glycogen.**

a. To an extract of liver (prepared by extracting the perfectly fresh organ with boiling water after washing) add a solution of iodine in potassic iodide. The liquid assumes a red colour identical with that yielded under similar circumstances by dextrine (*See* I., 5).

b. Repeat 4, substituting extract of liver for starch paste, using the same precautions.

VI.—Bile.

1. Observe colour and reaction of ox bile. It is usually brown. Neutralize and boil in a test-tube. Bile does not contain albumin.

2. Add a few drops of bile to methylated spirit. **Mucin** is precipitated.

3. Prepare a solution of syntonin (III., 4) by digesting albumin in water containing 0·2 per cent. of hydrochloric acid. On the addition of a drop of bile, the mixture curdles *en masse*. If a large quantity of bile be added, little or no precipitate may be formed, the liquid being rendered alkaline.

4. Boil bile with three-times its bulk of strong hydrochloric acid for ten minutes. The bile is decomposed into bile-resin (cholic acid with colouring matter) and glycin and taurin, the two last-mentioned substances remaining in solution.

5. **Pettenkofer's Test** for **Cholic acid.**—Spread a drop of bile in a thin film on a white porcelain capsule. Mix with a drop of strong solution of cane-sugar. Add concentrated sulphuric acid drop by drop, and, if necessary, warm. A deep purplish-red colour appears.

6. Repeat the test with an alcoholic solution of **bilin.** The same colour is produced.

7. **Gmelin's Test** for the **colouring matter.** Spread a drop of bile in a thin film on a white porcelain capsule. Allow a drop of strong nitric acid to fall into the middle of the film and observe the effect. The drop becomes surrounded by rings of green, blue, red, and yellow, in the order in which they have been named. Consequently, the

green, which is first formed, is eventually farthest from the drop of acid. If, instead of allowing the liquid to remain undisturbed, the acid be mixed with the bile, the liquid passes through the same tints, in the same order.

8. Warm a little nitric acid in a test tube. Incline the tube and pour bile down the side, so as to form a layer over the acid. The colours appear as in 7, at the line of contact of the two liquids.

9. **Cholesterin.** An ethereal extract of gall stones yields, on evaporation, crystals of cholesterin, which, when dropped into warm sulphuric acid, dissolve with a red colour. The residue, insoluble in ether, consists of colouring matter and mucin.

VII.—Urine. (1)

1. Observe reaction and colour.

2. Determine the specific gravity either by weighing or with the urinometer. Observe the effect of temperature.

3. Compare fresh with stale urine as regards appearance, smell, and reaction.

4. **Sulphates.** Add baric chloride after acidifying with hydrochloric acid. A white precipitate of baric sulphate is formed.

5. **Chlorides.** Add argentic nitrate after acidifying with nitric acid. A white curdy precipitate of argentic chloride is produced.

6. **Phosphates.** Add ammonic molybdate to urine which has been mixed with half its volume of nitric acid. Boil. A yellow crystalline precipitate falls.

7. **Urea.** To urine evaporated to one-third, add a drop of nitric acid in a watch-glass. Glistening scales of urea nitrate are abundantly formed in the liquid.

8. **Uric Acid.** To a hundred c.c. of urine add 5 c.c. of strong hydrochloric acid. Allow the liquid to stand for forty-eight hours. Dark red crystals of uric acid separate from the liquid.

9. **Urochrome.** Precipitate about 50 c.c. with lead acetate and a drop of ammonia. Filter. The filtrate is colourless. Scrape the precipitate from the filter paper into a capsule. Mix with a few drops of strong sulphuric acid and add to the pasty mass a little alcohol. Filter. The yellow filtrate, on boiling with excess of strong sulphuric acid, turns black. Dilute the acid liquid with a large quantity of water. The **uromelanine** which separates in flocks is characterized by its extreme solubility in ammonia. It can be precipitated from its solution in ammonia by sulphuric acid.

10. **Indigo.** To 500 c.c. of urine add 250 c.c. of pure hydrochloric acid. Allow the liquid to stand twenty-four hours. A coppery scum floats on the surface. Filter. Treat the filter first with ammonia to extract the uromelanine, secondly with cold alcohol, which acquires thereby a red colour. On boiling the residue in alcohol, a blue solution is obtained, which exhibits the absorption spectrum of indigo-blue.

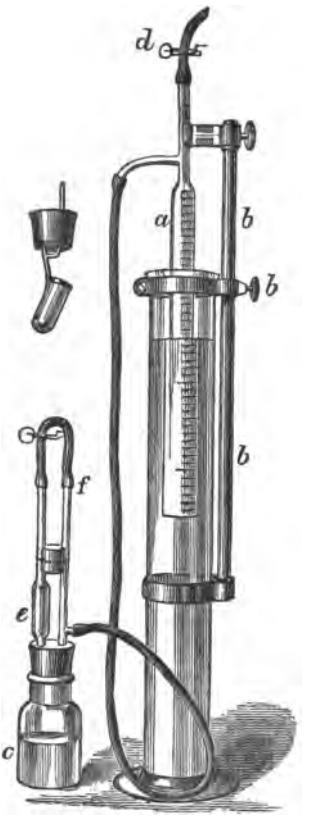
N.B.—In consequence of the large quantities which must be used, this experiment cannot be carried out by each student.

VIII.—Urine. (2)

1. **Quantitative determination of Urea. Urea** ($CO N_2H_4$) when decomposed by suitable oxidizing agents, yields CO_2 H_2O and N. The most convenient reagent for effecting this decomposition is an alkaline solution of sodic hypobromite. The CO_2 is absorbed by caustic soda. The nitrogen which is disengaged is collected and measured in a suitable ap-

paratus. Every 37·3 c.c. of nitrogen, at ordinary pressure and temperature, corresponds to 0·1 grm. of urea. The hypobromite solution is prepared by adding 25 c.c. of bromine to 250 c.c. of a solution containing 100 grms. of caustic soda.

FIG. 5.



The stopper and test-tube represented in the upper left hand of the figure takes the place of the stopper, pipette and tube *e f*. The woodcut has been kindly lent by Dr. Dupré.

Dupré's apparatus is used. Introduce 25 c.c. of hypo-

bromite into the flask *c*. Measure off 5 c.c. of urine into the test-tube, and close the flask with the caoutchouc stopper to which the test tube is attached. Open the pinch-cock *d* and lower the measuring tube *a*, until the surface of the water is at the zero point of the graduation. Close the pinch-cock and raise the measuring tube. If the apparatus be tight, mix the urine gradually with hypobromite solution by inclining the flask. Finally, tilt the flask so as to rinse out the test-tube with the solution, and shake well for a few seconds. Immerse the flask in a vessel containing water at the same temperature as that in the jar. At the same time lower the measuring tube. After two or three minutes, raise the measuring tube again until the surfaces of the liquids inside and out coincide. Read off the quantity of nitrogen which results from the decomposition of the 5 c.c. of urine.

2. **Quantitative determination of Phosphates.**—When solution of uranic nitrate or acetate is added in successive quantities to a hot solution containing phosphates, previously acidified with acetic acid, the whole of the uranium is precipitated so long as any phosphate remains in solution as uranic phosphate. As soon as an excess of uranic salt is present, it can be detected by potassic ferrocyanide, which gives a brown colour with uranic salts.

The standard uranic nitrate solution contains 35·5 grammes in a litre. One c.c. corresponds to 0·005 gramme P_2O_5.

To 50 c.c. of urine add 5 c.c. of a solution containing 100 grammes of sodic acetate in 900 c.c. of water, to which 100 c.c. of glacial acetic acid have been added. Heat the 55 c.c. to 80° C. Add the uranic nitrate solution, until a drop of the mixture placed on a white porcelain slab gives a distinct brown colour, with a drop of potassic ferrocyanide. Note

the quantity of solution used, and calculate therefrom the percentage of P_2O_5 in the urine *

IX.—The Colouring Matter of the Blood.

1. Observe the solar spectrum, noting the positions of the dark lines D, E, *b* and F, in relation to the colours. Compare it with the spectrum of a gas flame, which shows no dark lines.

2. Observe the spectrum of a flame coloured with sodic chloride, noting the position of the bright yellow line.

3. **Oxy-hæmoglobin.**—Introduce defibrinated blood into a test-tube, and observe its opacity when undiluted.

(a) Dilute by adding five to ten times its bulk of water. Place the test-tube in front of the slit of the spectroscope, direct it to a gas flame. The only light which passes through is that of the red end of the spectrum.

(b) Add water until the green appears. Note the dark space **(absorption band)** between the red and green.

(c) Dilute still further until the yellow-green light is distinguishable in the middle of the dark space, dividing the single broad band into two.

(d) After a further addition of water, note that the band nearest the D line is somewhat more sharply defined than the

* For details as to the hypobromite method, see Dupré's original paper in the *Journal of the Chemical Society*, 1877, vol. i. p. 534.

The method for the determination of P_2O_5 is practised in this class as an example of a volumetric process. For other methods relating to the urine see Part IV. It is important to remember, that in order to obtain trustworthy results, as scrupulous care must be taken in the measurement and collection of the urine passed during the period of observation as in the analytical procedures.

other. The spectrum is still shortened by the absorption of its violet end.

(e) On diluting, until the solution is almost colourless, two faint bands are still visible.

(f) Map on the diagram the appearances observed in 3, *b* and *d*.

4. **Reduced Hæmoglobin.**—To some blood diluted as in 3, *d*, add a drop of solution of ammonic sulphide, and warm gently. The colour becomes purplish. Place the tube in front of the slit as before, and observe the change which has occurred. A single absorption band, with ill-defined edges, takes the place of the two bands previously observed. Map its position on the diagram.

5. **Alkaline Hæmatin (Hæmochromogen).**—Add to solution of blood, rather stronger than the last, a drop of solution of caustic potash. Warm gently; the colour completely changes. An absorption band appears to the left of the line D, and much of the blue end of the spectrum is cut off.

6. **Reduced Alkaline Hæmatin.**—To the solution obtained in 5 add a drop or two of ammonic sulphide and warm gently. Observe the change of colour. Dilute if necessary. A strongly marked band is seen to the right side of the line D, and a second less defined, which nearly coincides with the line E.

7. **CO-hæmoglobin.**—Blood which has been acted upon by carbonic oxide has a peculiar cherry-red colour. The two absorption bands have nearly the same position as those of Oxy-hæmoglobin, but no change is produced when the liquid is treated with reducing agents, as in 4.

Part IV.

LABORATORY EXERCISES IN CHEMICAL PHYSIOLOGY.

I.—Starch and its Derivatives.

1. Prepare potato starch by grating potatoes into water, stir the mixture thoroughly, and allow it to stand. After partial subsidence pour off the turbid liquid and set it aside. Collect the white deposit of starch, mix it with a fresh quantity of water, and again separate it by subsidence and decantation.

2. Examine the product microscopically. Each granule exhibits concentric markings, which become more distinct after the partial action of solution of iodine. In the dark field of the polarisation microscope, each shows a dark cross on a bright area.

Digest starch in saliva at 45°—50° C. for 3 hours. Examine the insoluble residue of cellulose before and after treating with solution of iodine.

3. Boil a portion of the deposit in water to obtain "starch solution" and filter. Observe that the liquid is opalescent and that when a beam of sunlight passes through it the beam is luminous. When viewed transversely through a Nicol's prism, held between the thumb and forefinger, and rotated, the luminosity appears and disappears alternately. Compare in this respect solution of sulphate of quinine. Starch solution is indiffusible.

4. Grind some malt in a coffee mill, extract the meal with

four or five times its volume of water at 30° C. Concentrate the extract at the same temperature to one half.

5. Prepare some half per cent. solution of starch, to part of it add one tenth of its volume of malt extract and place the mixture in the warm chamber at 70° C. From time to time test portions of the liquid with solution of iodine. As soon as the liquid remains colourless after the addition of the iodine, arrest further change by boiling. Concentrate some of the product and pour it into an excess of alcohol. The precipitate is achroodextrin, maltose remaining in solution. Filter, evaporate the filtrate, re-dissolve the residue in water, and prove the presence of a reducing sugar.

6. Treat the remainder of the half per cent. solution of starch, with one-tenth of its volume of malt extract as before, keeping it in the warm chamber for three hours. Divide the product into two exactly equal parts; in one determine the reducing power by the volumetric method described below. Add to the other, two per cent. of concentrated sulphuric acid, boil for half an hour in a flask; bring the liquid to its original volume by the addition of water, and estimate the reducing power by the same method.

7. Volumetric estimation of sugar by Fehling's method.

Dissolve 34·639 grm. of pure cupric sulphate in about 200 c.c. of distilled water, also dissolve 173 grm. of pure double tartrate of potassium and sodium (Rochelle Salt) in 500 to 600 c.c. of a solution of caustic soda of 1·12 sp. gr., mix the two solutions and dilute to a litre. 10 c.c. of the solution so prepared are equivalent to ·05 grm. sugar.

Dilute 10 c.c. of the above solution with 40 or 50 c.c. of water, and heat to boiling in a white porcelain basin. Now run in from a burette the sugar solution (previously diluted so as not to contain more than one half per cent.

of sugar) and continue to do so until the blue colour of the copper solution disappears, *i.e.* until all the copper is reduced. Read the burette; the quantity used contains ·05 grm. of sugar, whence the total quantity and the amount of dilution being known, the total quantity of sugar may be easily calculated.

II.—Milk.

1. Determine the sp. gr. of a sample of new milk with the hydrometer. Allow it to stand for a day and skim off the cream and determine the sp. gr. of the skimmed milk. Add water until it is reduced to the original sp. gr. Ascertain what proportion of water is required for this purpose.

2. **Curdling of Milk.**

(*a*) **By Rennet.**—Warm 50 c.c. of milk to 45° C. and add 20 drops of commercial extract of rennet. Set it aside for ten or twenty minutes; the coagulum resembles that of blood in consistence; filter and preserve the whey.

(*b*) **By Acid.**—Dilute milk with eight volumes of water at 40° C., add a drop or two of acetic acid. Separate the curd which forms by gentle shaking, and filter; keep the filtrate.

Take two portions of the curd and dissolve one in weak caustic soda, and the other in lime water. Filter both from undissolved particles, and add a little rennet to both filtrates. The solution in lime water will coagulate, that in soda will not.

(*c*) **Salt Solution Curd.**—Treat milk with twice its volume of a saturated solution of common salt with the addition of powdered salt, and shake thoroughly. (This is best effected by a machine; it can, however, be done on a small scale by shaking milk in a test tube with powdered salt). The milk coagulates, and a clear filtrate free from butter and casein may be

obtained. Test the filtrate for sugar and coagulable proteids and estimate the former by the method already given.

Dissolve some of the curd in water and add rennet to the solution, observe that it coagulates.

It is obvious from these experiments that there are important differences between acid and rennet curd, and that calcium salts are accessory to the action of rennet.

(d) Take the whey from two equal quantities of milk, one prepared with rennet the other with acid. Determine the sp. gr. of each, and the amount of proteids. Compare the results carefully.

(*e*) Neutralize and boil acid or sweet whey. Filter and concentrate. Crystals of milk sugar may be obtained.

(f) Shake milk which has stood for several weeks in the warm chamber, and is strongly acid, with ether, and decant the ethereal extract, allow the ether to evaporate, and exhaust the residue with water. The watery extract is strongly acid, and has the characteristic sour smell of lactic acid.

(g) **Volatile Acids of Butter.**—Take about 5 grms. of butter, and treat it with excess of strong solution of caustic potash in alcohol, warming slightly. When the action is complete drive off the alcohol in the water bath and exhaust the residue with a little water, place the watery extract in a retort with excess of phosphoric acid and distil gently. Collect the distillate and observe its odour and reaction.

III.—Albumen of Egg and Serum.

1. Method of determining the **temperature of coagulation.**

"A glass beaker containing water is placed within a second larger beaker also containing water, the two being separated by a ring or cork. Into the water contained in the inner beaker there is immersed a test tube, in which is fixed an

accurately graduated thermometer, provided with a long narrow bulb. The solution of the proteid of which the temperature of coagulation is to be determined, is placed in the test tube, the quantity being just sufficient to cover the thermometer bulb. The whole apparatus is then gradually heated. With the arrangement described the rise in temperature takes place very slowly and equally throughout. Care being taken to have as good illumination as possible (the best plan being to place the apparatus between the operator and a well lighted window) the experimenter notes the temperature at which the liquid first shows signs of opalescence; he afterwards notes again the temperature at which a distinct separation of flocculent matter occurs."—Gamgee's *Physiol. Chem.*, vol. i., p. 15.

2. Dissolve albumen in twenty times its volume of ten per cent. salt solution. Introduce some of the liquid into a loop of dialysing tube (of parchment paper), previously tested by filling it with water, and suspend the tube in a beaker of water. Test the external liquid from time to time for chlorine, by the following process:—

3. **Estimation of Chlorine in the Diffusate**:—Dissolve 3·44 grms. of pure fused silver nitrate in distilled water, and make up the solution to a litre. Dissolve also 1 grm. of pure fused sodium chloride in water, and dilute to one litre. Fill a burette with the silver solution, measure with a pipette, ten c.c. of the sodium chloride solution, and place it in a beaker with one drop of a solution of neutral potassic chromate. Now run in the silver solution, cautiously stirring the mixture during the process, until the red colour produced by each drop of the silver solution, and which at first disappears on stirring, becomes permanent. If the solutions have been accurately prepared, ten c.c. of the silver solution corresponding to ·01 grm. sodium chloride will be required.

Construct a dialysing cell by closing the ends of a length of dialysing tube with india-rubber corks, one of which is perforated for two glass tubes. Of the two tubes the longer must reach to the lower cork and be bevelled at its end; the other must be cut off short. Through this arrangement send a current of water from the tap. Fill a narrow cylindrical vessel, not much larger than the tube, with serum, and plunge the latter into it. Allow the water to flow for 12 hours and observe the precipitation of the globulin.

4. Prepare Lieberkühn's jelly, by adding strong potash solution drop by drop to undiluted white of egg, stirring vigorously; cut up the jelly into small pieces, and throw it into a large beaker kept full of water by an arrangement similar to that described in 3. Keep the produce in water for further use.

5. Prepare the whites of two eggs, disintegrate them by clipping with scissors, rinse with twenty parts of water by volume. Introduce the mixture into a corked flask, which must not be more than half full, and shake briskly. Strain through muslin and filter.

6. Dilute serum of blood with water, so that the mixture shall have as nearly as possible the same sp. gr. as the previous one (5).

Compare the properties of the two forms of albumin, according to the following table:—

Serum albumin	Egg albumin
Serum albumin is coagulated between 60° C. and 75° C. It is not readily precipitated by hydrochloric acid. The precipitate is readily soluble in excess.	Egg albumin is coagulated between 63° C. and 70° C. It is readily precipitated by hydrochloric acid. The precipitate is *not* readily soluble in excess.
The precipitate, by boiling, is readily soluble in strong nitric acid. Its solution is not coagulated by ether.	The precipitate, by boiling, is soluble with difficulty in strong nitric acid. Its solution is coagulated by ether (if the solution be not alkaline).

IV.—Globulin of Serum.

1. **Paraglobulin or Fibrinoplastin.**—When solid magnesium sulphate is added in large excess to serum, and the mixture violently agitated for some time by mechanical means, a dense precipitate is obtained (serum globulin). This body is an example of a class of proteids which are distinguished by being insoluble in water, soluble in weak solutions of neutral salts, but insoluble in saturated ones.

2. Collect the liquid in which the globulin has been precipitated, as above described, on several filters. It will require many hours for filtration. Preserve the filtrate.

3. After the filtration is complete, wash the filters with as little water as possible, this will rapidly dissolve the precipitate. The apparent solubility is due to the presence of magnesium sulphate.

4. Add excess of magnesium sulphate in powder to the clear filtrate from (2). It becomes turbid when shaken for some time.

5. Test the coagulation point of the liquid as before directed.

The Older Methods of Separating Serum Globulin.

6. **Panum's method:**—Dilute serum with fifteen times its bulk of water, and add four drops of twenty-five per cent, acetic acid to every 160 c.c. of the mixture.

7. **Alex. Schmidt's method:**—Dilute serum with twenty volumes of water, and pass a stream of CO_2 through the mixture, keeping it cool during the process.

8. Collect the precipitate on a filter and wash it with water saturated with CO_2. It is insoluble in water that has not

been boiled; in boiled water it dissolves with great difficulty.

N.B.—An additional precipitate may be obtained from the filtrate, by treating it with a trace of acetic acid. This was formerly regarded as serum casein by Panum.

9. Add to the filtrate from 3, sodium sulphate in powder in excess, a further precipitate is obtained. This is serum albumin. The filtrate still contains a small quantity of a substance which gives the proteid reactions.

10. **Properties of Pericardial Fluid**:—Obtain pericardial fluid from the horse slaughterers, remove the contents of the pericardium with a clean glass syringe, choosing for the purpose an emaciated animal, and taking the greatest possible care to avoid admixture with blood. The liquid should be clear, and of a pale amber colour.

11. If freshly collected, it either fails to coagulate, or coagulates very tardily. Whether it coagulates or not, it yields when treated as in 1 a large precipitate of globulin similar to that of serum.

N.B.—If hydrocele fluid can be obtained, it may be used instead of pericardial fluid.

12. **Coagulation of Blood-Plasma**;—Prepare magnesium sulphate plasma, by receiving two parts of horse's blood into one part of a saturated solution of magnesium sulphate. Great care should be taken to mix the liquids thoroughly immediately. The mixture must stand in a cool place (preferably in ice) for two days, to settle. After subsidence, the supernatant liquid must be carefully drawn off with a pipette.

13. Collect a quart of horse's blood in a wide-mouthed stoppered bottle, and set it in ice immediately. After it has been allowed to stand for two days, draw off the serum, and strip off the upper colourless stratum of the clot (buffy coat), and place it in salt solution.

14. Dilute some of the magnesium sulphate plasma with fifteen to twenty volumes of water. Place it in the warm chamber at 35° C. It coagulates.

15. Mix serum with pericardial fluid in about equal volumes, and treat the mixture as in 14.

16. Add to pericardial fluid in a watch-glass, a fragment of fresh buffy coat, and place it in the warm chamber. Coagulation occurs.

V.—Digestion.

Natural Digestion.—The state of the digestive organs during the process of digestion, can be best seen in a dog which has been well fed with meat freed from masses of fat some seven hours previously. The animal may be conveniently killed by the injection of cyanide of potassium into the pleural cavity.

1. Open the abdominal cavity freely, in order to examine the condition of the lacteals and thoracic duct. Tie the stomach off from the duodenum by two ligatures, dividing between them; do the same at the lower end of the ileum. Open the stomach along the greater curvature, turn it inside out, and collect the semi-solid pulp which fills it. Observe that there is very little liquid. Dilute the contents to 250 c.c. with water (if the dog is of moderate size), strain through muslin, and subsequently filter, precipitate the acid liquid by boiling with ferric acetate. (This may be prepared by precipitating a solution of iron alum with lead acetate, allowing it to stand overnight, and decanting the clear liquid). Filter and test the filtrate for peptone, with alkaline solution of cupric hydrate. The solution should give no precipitate with potassic

ferrocyanide and acetic acid. The same method may be applied to the contents of the intestine. Note the absence of foul odour and (under the microscope) of bacteria.

2. **Artificial Digestive Products.—Gastric Digestion.**—Place a portion of mucous membrane scraped off the stomach in a beaker, with 0·2 per cent. hydrochloric acid at 38° C. in the warm chamber. Observe its rapid disappearance, filter the liquid, and test its digestive power by the method described in 7.

3. Make a five per cent. solution by volume of white of egg in 0·2 per cent. hydrochloric acid, add to it glycerine extract of stomach in the proportion of 10 c.c. to the litre. Leave it in the warm chamber for twelve hours; neutralize, separate the neutralization precipitate by filtration, boil the filtrate with ferric acetate as before directed, filter and test the filtrate for peptone as before.

4. **Pancreatic Digestion.**—Test the activity of the glycerine extract of pancreas by the method described in Part III., IV.

5. Pound part of an ox's pancreas into a pulp, place it in one per cent. solution of sodium carbonate in the warm chamber, and allow the product to remain in the warm chamber for several days. Strain some of it through muslin, filter, boil the filtrate, and again filter. Boil the second filtrate with Millon's reagent.

6. Apply the same process to the extract of the intestinal contents, and compare the results.

7. **Grützner's Method of Comparing the Digestive Powers of Solutions.**—This depends upon the fact that when fibrin stained with carmine is subjected to the action of a digestive ferment, as the fibrin is dissolved, the carmine is liberated and colours the fluid, the amount so set free being estimated

by an artificial scale consisting of ten solutions of carmine of different strengths, equal quantities of the stained fibrin being mixed with equal volumes of the digestive liquid to be compared.

The following materials must be prepared:—

1. **Carmine solution for staining the fibrin**; take 1 grm. carmine and dissolve it in about 1 c.c. of strong ammonia, add 400 c.c. of water, place it in a bottle loosely stoppered until the smell of ammonia has become faint. 2. **Stained fibrin**; take perfectly fresh and clean fibrin, chop it very fine, place it in carmine solution for twenty-four hours, strain off the fluid, and wash in water until the washings are colourless, squeeze out the excess of fluid, and keep in a stoppered bottle with just enough ether to cover it. 3. **Another solution of carmine to serve as a standard of colour.** This is made by dissolving 1 grm. of carmine in ammonia as before, and dilute with glycerine to 100 c.c.

Take 6 c.c. of solution 3, and dilute to 60 c.c. with water. Charge ten test tubes with quantities of the mixture ranging from 1 to 10 cub. centims., and dilute each with water to 20 c.c. Cork the tubes and number them in order, according to the strength of the solution it contains.

With the aid of a measure to hold 1 c.c., which can be made by closing one end of a piece of glass tubing, and grinding the open end, measure out two equal quantities of stained fibrin, and place them in two test tubes A and B, each of which contains 20 c.c. of 0·2 per cent. hydrochloric acid. To A add a measured quantity of one digestive liquid, and to B precisely the same quantity of the liquid with which it is to be compared. Place both in the warm chamber, and at regular intervals, which must be carefully noted, compare each (after shaking) with the colour scale.

VI.—Urine.

1. **Preparation of Urea from Urine.**—Evaporate the urine to a small bulk. Add strong nitric acid (pure and free from other oxides of nitrogen) in excess, keeping the mixture cool during the addition of the acid. Pour off the excess of fluid from the crystals of urea nitrate which are formed, set them on a porous tile or perforated dish to drain, press as much fluid from them as is possible. Add barium carbonate in large excess, mixing thoroughly, dry on a water bath and extract with absolute alcohol; filter, evaporate the filtrate to a syrup on the water bath, and set aside to crystallize. The product may be further purified by animal charcoal and recrystallization. In order to carry out this process properly, a whole day is required.

2. A quicker method is the following. Take 20 c.c. of urine. Add "baryta mixture," (two volumes of barium hydrate solution, and one volume barium nitrate solution, both saturated in the cold), until no further precipitate is produced; filter, evaporate to a thick syrup on the water bath, and extract with alcohol. Pour off the alcoholic extract, filter, and again evaporate to dryness on the water bath, and take up with water. Place a drop of the watery solution on each of the two slides, add to one, strong nitric acid; allow both to crystallize, and examine under the microscope.

3. **Biuret Reaction.**—Heat urea to 150°-160° C. Dissolve the product in a little warm water and add a solution of cupric sulphate in caustic potash; a characteristic violet tint is produced.

3. **Dupré's Method.** (See Part III., VIII.)—Urea, when acted upon by alkaline hypobromites or red fuming nitric

acid, splits up into carbonic anhydride, nitrogen, and water. This decomposition forms the basis of Dupré's and other methods of estimating urea by measuring the amount of nitrogen produced in the reaction. In this method, only 92 per cent. of the nitrogen of the urea is given off. Uric acid yields less than half, the other nitrogenous constituents of the urine, variable proportions of their nitrogen. Hippuric acid is not decomposed. In the graduation of Dupré's apparatus this error is taken into account.

5. **Liebig's Method.**—A solution of pure mercuric nitrate having been prepared, of such a strength that 20 c.c. of it are required for the precipitation of 10 c.c. of a 2 per cent. solution of urea,—of which, therefore, 10 c.c. correspond to 0·1 grm. of urea;—add 20 c.c. of the baryta mixture (see 2) to 40 c.c. of urine, and filter. Measure 15 c.c. of the filtrate, which represents 10 c.c. of urine. Place it in a beaker, and run in the mercury solution, (each c.c. of which corresponds to 0·01 grm. of urea), until on mixing a drop of the mixture with a drop of a saturated solution of sodium carbonate on a white tile, a pale lemon colour appears. Now read the burette, and calculate as follows:—If 10 c.c. of urine contained 0.2 grm. of urea, it would require 20 c.c. of the mercury solution, *i.e.*, 1 c.c. for each grm. per litre. If the daily quantity of urine were 1500 c.c. this would give a daily discharge of 30 grms. of urea.

This method approaches accuracy only when the quantity of urea present is about 2 per cent. The chlorine in the urine must also be estimated, and the quantity of urea indicated reduced by the subtraction of 1 gramme of urea for every 1·3 grms. of sodium chloride found.

6. **Estimation of Chlorides by Liebig's Method.**—The following solutions must be carefully prepared. *a.* A solution,

of mercuric nitrate of such a strength that 1 c.c. shall correspond to ·01 grm. of sodium chloride. Dissolve 20 grms. of pure mercury in boiling nitric acid, until a drop of the acid fluid does not cause a precipitate when added to a solution of common salt; then dilute to nearly a litre. *b.* A solution of pure sodium chloride, 20 grms. to the litre. *c.* A solution made by dissolving 4 grms. of pure urea in 100 c.c. of diluted water. *d.* A solution of sodium sulphate saturated in the cold.

To prepare the standard mercuric nitrate solution, place 10 c.c. of the standard sodium chloride solution in a beaker, together with 2 c.c. of the urea solution, and 5 c.c. of the solution of sodium sulphate. Now allow the solution of mercuric nitrate to flow gently into the beaker from a burette (with glass tap), stirring the mixture during the process. A precipitate forms at first, and is redissolved on stirring. On adding more of the mercurial solution, the fluid becomes opalescent, but no permanent precipitate forms until the reaction is complete. The strength of the mercurial solution having been determined by several experiments, it is diluted so that 20 c.c. = 0·2 grm. sodium chloride = 10 c.c. of the standard sodium chloride solution.

Precipitate the urine with baryta mixture as directed in 5. Take 15 c.c. of the filtrate, make it *very slightly* acid with very dilute nitric acid, and then run in the mercury solution cautiously, until a permanent dense cloud, not affected by vigorous stirring, makes its appearance; the number of c.c. used, multiplied by 0·01, gives the amount of chlorine as sodium chloride contained in 10 c.c. of urine.

This process depends upon the fact that when mercuric nitrate and alkaline chloride in solution are mixed, sodium nitrate and mercuric chloride are formed. It is, therefore,

not until all the chloride in the urine has been so decomposed, that mercuric nitrate begins to combine with the urea present, to form a permanent white precipitate. The necessity for estimating the chlorides when using Liebig's process for the determination of the urea is obvious.

7. Estimate the sugar in a sample of diabetic urine by the process already given in I. 7.

8. **Uric Acid.**—Boil serpent's excrement with a 10 per cent. solution of caustic soda. Dilute, allow the liquid to stand, and decant the clear fluid and pour it into a large quantity of water to which 10 per cent. by volume of hydrochloric acid has been added. Allow it to stand twenty-four hours, wash and preserve the crystals which are deposited.

9. Treat uric acid or a urate in a porcelain dish with dilute nitric acid, and evaporate to dryness; after cooling allow a drop of strong potash to run over the residue. A deep purple colour is produced.

10. **Hippuric Acid.**—Take 200 c.c. of fresh cow's urine and concentrate it on the water bath to 110 c.c., add Hydrochloric acid and set aside till the next day. Take the brown crystalline mass which forms, wash with cold water, press between folds of filtering paper, dissolve in as little boiling water as possible, add a little pure animal charcoal and filter, concentrate the filtrate and set aside to crystallize.

11. To obtain hippuric acid from human urine take (soon after a good meal) 1 to 1·5 grms. of benzoic acid in wafer paper. Collect the urine of the rest of the day and following morning. Evaporate to a syrup on the water bath, extract with alcohol.

12. Dry some of the crystals obtained by the method above given, 11, between folds of filter paper and treat in a test tube, observe that a red oil forms, and that ammonia and

benzoic acid are given off as may be recognized by the smell.

13. **Creatinin.**—Precipitate 200 c.c. of urine with milk of lime. Filter and evaporate to a syrup. Extract with large excess of alcohol and filter. Add to the filtrate two drops of a perfectly neutral solution of zinc chloride. Set the liquid aside in a dark, cool place for two or three days. Creatinin zinc chloride crystallizes in rosettes, in vertical lines on the sides of the vessel.

VII.—Urinary Deposit.

Urinary deposits may be divided into two classes, according to the reaction of the urine in which they occur.

1. **Deposit in acid urine.**—(*a*) The so-called lateritious deposit which forms on cooling in clear healthy urine, of high specific gravity, and acid reaction, consists chiefly of uric acid and urate of sodium, coloured by the urinary pigments. This deposit may be obtained from any acid urine by slightly concentrating and allowing it to stand.

Allow the deposit to subside and decant the liquid from it. Examine it under the microscope. If uric acid is present its characters can readily be recognized. Separate the crystals from the amorphous deposit of urates, by washing with water, and decantation. Note that the urate is readily soluble in warm water, especially if it contains a trace of alkali.

(*b*) Calcic oxalate is also deposited under certain conditions, in acid urine. It may be readily recognized by the microscope. To observe its characters add to warm urine a few drops of solution of calcic chloride and not more ammonium oxalate than is sufficient to produce a very slight precipitate.

Allow the urine to stand over night. After the ingestion of any considerable quantity of rhubarb, calcic oxalate is always deposited.

2. **Deposits in alkaline urine.**—(*a*) Add to ordinary urine a few drops of urine which is already ammoniacal, and allow it to stand for a few days in a warm place.

The deposit is whitish and consists of amorphous basic phosphates of calcium and magnesium, identical with the amorphous precipitate which is thrown down when urine is neutralized—along with which are seen crystals of phosphate of ammonium and magnesium (triple phosphate) and minute organisms, on the surface of the urine is a scum which contains the same crystalline and organized forms.

(*b*) Filter ammoniacal urine (previously decanted from the sediment) wash the filter and dry it, then dip it in alcoholic solution of turmeric and dry. The paper if wetted with fresh urine and exposed to a current of air becomes brown, owing to the decomposition of the urea.

c. Add to urine ammonium chloride and traces of sodium phosphate and magnesium sulphate. Urine thus treated yields on addition of ammonia a precipitate which on standing becomes crystalline. Compare the crystals with those obtained from ammoniacal urine.

d. Acidulate urine with acetic acid, then add calcium chloride and sodium phosphate in relatively small quantities, A precipitate is formed, which becomes crystalline, of neutral calcic phosphate. This body is precipitated under similar conditions in urine.

VIII.—Muscle.

1. **Creatin,** from aqueous extract of muscle, or best, from Liebig's extract.—Dilute the latter with fifty times its volume of water and precipitate it with lead acetate, excess being avoided. Filter, separate the lead by sulphuretted hydrogen and again filter. Evaporate to small bulk and set aside for a week to crystallize. Pour off the mother liquor, and add three or four volumes of alcohol sp. gr. 0·982, filter, wash with alcohol, dissolve all the crystals obtained in boiling water, again filter and set aside to crystallize. Creatin crystallizes in transparent, colourless, rhombic prisms, for which those of common salt may be easily mistaken. They are distinguished from them by appearing illuminated in the dark field of the polarization microscope.

2. **Sarkin or Hypoxanthin.**—If the filtered alcoholic extract of the mother liquor of creatin be evaporated nearly to dryness on a water-bath, extracted with water and filtered, the filtrate yields on the addition of ammonia and ammoniacal solution of silver nitrate, a precipitate which consists of silver compounds of hypoxanthin and xanthin. For the method of obtaining these bodies, see Gamgee's *Physiological Chemistry*, p. 329.

3. **Acidification of Muscle.**—(*a*) Test the reaction of a fresh sectional surface of curarized muscle. For this purpose use two strips of glazed litmus paper of different colours. Place them edge to edge on a varnished board, and affix the cut surface over the junction. The reaction is neutral or feebly alkaline. Test it again half an hour later, it will be found to be acid, notwithstanding that the muscle has been kept in a moist atmosphere, or under mercury.

(*b*) Repeat the experiment, keeping the muscle at 45° C, observe that it becomes acid much sooner.

(*c*) Repeat (*a*) after subjecting the muscle to prolonged tetanus. This is accomplished by faradising the spinal cord in a decapitated frog, after previous division of the sciatic on one side, and comparing the reaction of the cut surface of the muscle which has been tetanized with that of the other.

4. The production of carbonic acid. That this process is dependent upon contraction may be shown by arranging two preparations of the hind limbs of a frog in closed vessels, over lime or baryta water. Of these, one is continuously tetanized, the other is not, and air (previously deprived of its CO_2 by passage through strong potash) is aspirated slowly through both. If the experiment be continued for some time, it is seen that much more carbonate of lime or baryta is deposited in the vessel containing the tetanized muscle than in the other.

IX.—Bile.

1. **Mucin.**—Add to bile diluted with an equal volume of water, excess of alcohol. Mucin is precipitated; filter, wash the precipitate with dilute spirit, and dissolve in lime water and again filter. Add excess of acetic acid to the filtrate. Test the alkaline solution of mucin with Millon's reagent, nitric acid, normal and basic lead acetate.

2. Preparation of **bile crystals.**—These consist of a mixture of glycocholate and taurocholate of sodium, and are to be prepared as follows:—evaporate the bile to a thick syrup, and extract with strong alcohol, decolorise the alcoholic extract with animal charcoal, filter and evaporate off the

alcohol on a water-bath, extract the residue with absolute alcohol, pour the solution into excess of ether, and set aside to crystallize in a well-stoppered bottle.

3. **Taurin.**—Boil dried or inspissated bile, or bile crystals, for some time with strong hydrochloric acid in a flask fitted with a long tube for condensing, filter, evaporate to dryness, extract with absolute alcohol, dissolve the residue in water, and set aside to crystallize.

4. **Colouring matters.**—(*a*) Take dog's bile, acidulate with acetic acid, add excess of chloroform, and warm gently; remove the chloroform with a pipette, and evaporate on a water-bath, the residue is crude bilirubin.

(*b*) Extract gall-stones with ether. Keep the ethereal extract for the cholesterin it contains. Allow a few drops to crystallize on a slide. Examine the crystals under the microscope, and apply the iodine and sulphuric acid test. Boil the residue in water acidulated with hydrochloric acid; pour off the bulk of the fluid, and add warm chloroform. The solution on evaporation yields crude bilirubin. Test the solubility of the product in water, ether, alcohol, and chloroform. Examine the solution before the spectroscope, and observe that it shows no absorption bands.

X.—Glycogen of the Liver.

1. Feed a rabbit on carrots, and, five or six hours afterwards, kill it by opening the carotids. Open the chest and abdomen quickly. Insert cannulæ into the vena portæ and vena cava superior respectively, and pass a gentle stream of water through the liver until it becomes uniformly pale. Now cut it out quickly, mince it, and throw the pieces at once into

boiling water acidulated with acetic acid. Filter the liquid hot, evaporate to small bulk, and add large excess of alcohol. The glycogen is precipitated as a flocculent powder.

2. Take the livers of two or three oysters, mince finely, and throw the pieces into boiling acidulated water, or treat as in 1.

3. Dissolve a small quantity of glycogen prepared as above, in water, add a few drops of saliva, place the mixture in the warm chamber for five minutes, and test for sugar.

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